Induced ovulation and larval rearing of four species of Australian marine fish

by

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Submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy
University of Tasmania March, 1995

DECLARATION AND AUTHORITY OF ACCESS

I hereby declare that the material in this thesis is original except where due acknowledgement is given, and the material has not been accepted for the award of any other degree or diploma.

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Stephen Christopher Battaglene

ACKNOWLEDGMENTS

I sincerely thank my supervisor Dr B Nowak (University of Tasmania) and co-supervisor Dr S Rowland (NSW Fisheries) for their encouragement, friendship and advice throughout my candidature. I thank Dr R Kearney (Director, NSW Fisheries, Fisheries Research Institute) and Ms B Richardson (formerly, Deputy-Director NSW Fisheries) for their support, and the opportunity to enter the field of aquaculture research.

The research reported in this thesis was financially supported by NSW Fisheries, and the Fishing Industry Research and Development Corporation of Australia. I am very grateful for the support of NSW Fisheries, especially staff from the Port Stephens Research Centre (PSRC) and the Fisheries Research Institute (FRI). I thank successive PSRC managers Messrs R Martin, C Weale, S Griffin, I Embury and T Roberts for their support.

I am deeply grateful for the assistance, encouragement and constructive criticism given by Drs G Allan, J Bell, and J Nell (NSW Fisheries). I thank Drs P Gerhke, J Harris, M. Heasman, N Quartararo, Messrs S Fielder , W O' Connor, B Talbot (NSW Fisheries) and Ms S McBride, Dr G Maguire, Associate Professor N Pankhurst (University of Tasmania), Professor T Hecht (Rhodes University, South Africa), Mr R Orhun (San Diego State University, United States of America) and several anonymous referees for reviewing various manuscripts and making many useful comments. Dr J Paxton (Australian Museum) provided information on the taxonomy of fish. I thank Dr B Chatain (IFREMER, France), for sharing her knowledge of fish breeding and larval rearing.

To the staff of the PSRC, especially those working in the marine fish breeding program, my sincere thanks for all your assistance. I am indebted to Mr B Talbot (Senior Technical Officer PSRC) for his excellent technical assistance with broodstock collection, live food production, larval rearing, photography and experiments. I also thank Messrs P Beevers, M Lockhart, J Taylor and Miss J Cleary for routine hatchery production, data collection and assistance with experiments. Messrs W O'Connor and J Diemar kindly provided algae, and Mr R Balzer trucked in oceanic sea water. The workshop staff at PSRC built, modified and maintained the hatchery facilities; my thanks to all those involved, particularly Messrs R Balzer, W Bennet and C Morris. Mr P Beevers kindly assisted with data entry on computers and technical drawing, Ms J Pickles expertly typed the text and Mr D Reid (FRI) provided advice on statistical analyses.

Mr P Selosse (a former Technical Officer at PSRC) designed and conducted the early hormone induction trials with Australian bass, and kindly provided the data for inclusion in Chapter 2. Ms S McBride (University of Tasmania) and Mr G Le Berre (Montpellier University, France) assisted with sand whiting larval rearing trials and some experiments.

I thank the commercial fishermen of Port Stephens for providing broodstock, in particular, Mr I Walker for snapper broodstock. Mr G Searle (a Clarence River prawn farmer) provided ponds for extensive larval rearing trials and assisted in data collection. Mr L Hardup taught me how to catch small (<8 kg) mulloway, I'm still learning how to catch large broodstock.

All of the experiments presented in this thesis were designed and analysed by myself. I also wrote all of the scientific papers. Six of the Chapters (3-6 and 10-11) are co-authored by Mr B Talbot in recognition of the intellectual and technical contribution he made. Three other papers are co-authored, by Dr G Allan (Chapters 5 and 7) who presented one paper at an international conference in Singapore, by Mr P Selosse (Chapter 2) who provided some of the data and by Ms S McBride (Chapter 10) in recognition of her intellectual and technical contribution.

Finally to Jane, Lucy, Emma and Rosie my heart-felt thanks for your love and patience. Rosie you were born in the middle of Chapter 8, thank you for keeping me company late into the night.

Dedicated to Wendy Elizabeth Clare Sachs

ABSTRACT

This thesis developed techniques for the large-scale breeding of marine fish in New South Wales. It provides the first published account of the hormone induction and larval rearing to metamorphosis of Australian bass *Macquaria novemaculeata* (Percichthyidae), snapper *Pagrus auratus* (Sparidae), mulloway *Argyrosomus hololepidotus* (Sciaenidae) and sand whiting *Sillago ciliata* (Silliganidae) in Australia. Wild-caught broodstock were induced to spawn using hormones (hCG, LHRHa, Ovaprim) and either spawned naturally or were stripped. *Pagrus auratus* and *S. ciliata* were found to be multiple spawners with asynchronous ovaries. Conversely, *M. novemaculeata* and *A. hololepidotus* were found to be highly fecund single spawners having group synchronous ovaries. All four species were successfully induced to ovulate after periods in captivity ranging from one to five years. Species specific differences in hormone induction were determined, particularly in relation to the overripening of eggs and the optimum time between treatment and stripping.

Commercial scale batches of larvae were reared in 2000 L conical tanks and replicated experiments were conducted in aquaria ranging in size from 2 to 70 L. Larval development for all four species was described and egg size, time to hatch, size at hatch, yolk size, oil globule size, and beginning of exogenous feeding were compared among species. First feeding larvae were reared on rotifers *Brachionus plicatilis* and then brine shrimp *Artemia sp.*. Survival, growth and weaning of larvae at metamorphosis were compared.

Factors affecting larval survival, particularly those influencing initial swim bladder development were tested in replicated laboratory experiments. The timing of swim bladder inflation was found to coincide with the start of exogenous feeding but feeding was not required for initial swim bladder inflation. Larvae that failed to inflate their swim bladder grew poorly and were susceptible to stress induced mortality. Light intensity, was shown to be an important factor influencing swim bladder inflation in cultured larvae. The effect of light intensity on inflation in *M. novemaculeata* and *S. ciliata* was quantified. Exposure to continuous light (100-200 Lux) inhibited inflation in *M. novemaculeata*. In contrast, *S. ciliata* were shown to have a diel pattern of nocturnal inflation and required higher light intensities to feed (1000 Lux). Manipulative experiments with *S. ciliata* larvae showed that they responded to darkness by inflating their swim bladders. Other abiotic factors such as surface access, low salinity and high aeration were shown to reduce inflation in larvae. Initial swim bladder inflation strategies are discussed and recommendations made regarding the importance of maximising inflation.

The results of the study were used to assess the relative difficulty of intensive commercial production of each species for aquaculture.

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CHAPTER 1

GENERAL INTRODUCTION

1. POTENTIAL FOR MARINE FISH FARMING IN AUSTRALIA

The demand for fisheries products has grown over the past decade worldwide (FAO 1993; Treadwell et al. 1992). In Australia, the apparent consumption of fisheries products has increased from 15.1 kg per person in 1981-82 to 17.9 kg per person in 1989-90 (Treadwell and McKelvie 1991). This increase has been primarily supplied from wild fisheries and imports. Australian imports of fisheries products were estimated to be AUD$480 million in 1990-91 of which 52% were fin-fish (ABARE 1991). Rising incomes and increased public awareness of the dietary value of fish in the prevention of heart disease may have contributed to this demand (Treadwell et al. 1992; Sinclair and Anderson 1994).

Despite having the third largest fishing zone in the world, Australia's fisheries resources are not as abundant or productive as those in many other parts of the world (Williams and Stewart 1993). Furthermore, 31 species or groups are being heavily exploited by commercial and recreational fishermen and there are increasing restrictions on total catch (Williams and Stewart 1993). It is therefore not surprising that many sectors of the Australian fishing industry have a growing interest in aquaculture. In 1991-92, aquaculture production represented AUD$254.4 million or some 20% by value of the total Australian fisheries production (O'Sullivan 1995).

Marine fish farming is potentially an aquaculture growth area in Australia. However, a number of technical constraints currently limit its expansion. First, there is no reliable supply of juvenile fish or 'seed stock' for most species with aquaculture potential in Australia (Purser 1988; Stanley 1991; Treadwell et al. 1992). Seed supply is a fundamental requirement for fish farming and it is one of the most critical factors in the commercial success of the industrial production of marine fish (Shepherd and Bromage 1988). Second, the importation of exotic fish species into Australia is strictly controlled (Pollard 1989) and all new farming candidates will have to be Australian native species or exotic species like salmonids which have already been released in the wild. This restriction will hamper the development in the short term and is in contrast to the situation in Europe, Japan, and Israel, where the introduction of exotic species with aquaculture potential has been less restricted (Davy 1990; Sweetman 1992; Tandler 1993). Australian researchers will therefore have to select, breed and rear new endemic species for farming. The other constraints to marine fish farming in New South Wales and Australia which will need to be overcome include a scarcity of fish meal for marine feeds (Allan and Dall 1992) and the availability of suitable sheltered deep water cage culture sites.

The overall aim of the research reported in my thesis was to select native marine fish with aquaculture potential in the temperate waters of New South Wales and develop techniques for the large-scale breeding of four of these species.

2. STATUS OF AUSTRALIAN MARINE FISH FARMING

Fish hatcheries have been in existence in Australia since the 1860's. Exotic species like Atlantic salmon *Salmo salar* were produced at first (Faragher 1986), and later a wide range of native freshwater species were bred (Rowland et al. 1983; Cadwallader 1983; Burchmore and Battaglene 1986). However, the development of marine fin-fish farming in Australia has only occurred over the last decade with total production estimated to be about 5000 t in 1993, valued at AUD$72.5 million (D. O'Sullivan personal communication, 1995). Three species are commercially cultured: Atlantic salmon in Tasmania; southern blue fin tuna *Thunnus maccoyii,* in South Australia; and barramundi *Lates calcarifer,* in Queensland, South Australia and New South Wales.

Sea cage culture of Atlantic salmon started in Tasmania in 1987. It developed quickly due to the establishment of a large hatchery, the use of proven European hatchery technology, a high level of government involvement, excellent cage culture sites, and good water quality (Purser 1988; Treadwell et al. 1991). Production totalled 3300 t in 1993 at an estimated value of AUD$39.6 million (O'Sullivan 1993).

The farming of southern blue fin tuna started in 1989. The industry adapted the technology developed by salmon farmers in Tasmania. However, in contrast to salmon farming, tuna farming relies on the supply of fish from the wild. There is a limited number of wild fish available for on-growing and research is underway to breed tuna in captivity to allow increased production (Nicoll 1993). Tuna farming is profitable because of the high price paid (up to AUD$200/kg) for premium grade sashimi in Japan (Nicoll 1993). Production was estimated at 1000 t in 1993, valued at AUD$30 million (S. Clarke personal communication, 1994).

Barramundi were first bred in Australia in 1984 using intensive techniques developed in Asia (MacKinnon 1987). More recently, extensive larval rearing techniques in marine or brackish water ponds have been used (Rutledge and Rimmer 1991). Problems have been encountered with disease, cannibalism and declining market prices (Heasman 1989; Treadwell et al. 1991). However, the use of highly intensive shore-based growout systems (freshwater or saltwater) combined with a year-round supply of hatchery produced fish is currently increasing production (S. Fielder personal communication, 1995). Production was estimated at 255 t in 1993, valued at AUD$ 2.5 million (O'Sullivan 1993).

There is very little marine fish farming in New South Wales, although, it is the most populated state with the largest workforce, industry infrastructure and domestic fish market in Australia. Temperate conditions make it unsuitable for the culture of salmon or tropical species. Furthermore, the competition among recreational, industry and conservation groups for marine sites suitable for cage culture or intensive land-based sites with access to marine water has restricted development.

3. NEW SPECIES WITH AQUACULTURE POTENTIAL

Reviews listing marine fish with aquaculture potential in Australia and New Zealand are given by Maclean (1975), Pollock (1983), Garland (1988), Baker and Kenway (1989), Pankhurst and Pankhurst (1989) and Treadwell et al. (1992). Several studies have also presented economic models of farm profitability for new fish species (Cann 1990; Treadwell et al. 1991). New species have been chosen following consideration of the market potential, industry value, technical feasibility, production economics and compatibility with existing aquaculture industries (Treadwell et al.1992; Searle and Zacharin 1994). Research programs to determine the breeding requirements of newly selected marine species (Table 1), are currently established in all states and territories of Australia (Fig. 1), except the Australian Capital Territory and Victoria (Battaglene and Bell 1991; Searle and Zacharin 1994; O'Sullivan 1994).

In Tasmania, research has been conducted on rearing greenback flounder, *Rhombosolea tapirina* and long-snout flounder *Ammotretis rostratus* (Crawford 1984,1986). Greenback flounder are routinely produced by the Tasmanian Department of Primary Industry and University of Tasmania for assessment as a cage culture candidate (Hart et al. 1994). Other species being investigated in Tasmania are in the early experimental stage and include striped trumpeter *Latris lineata*, (Anon 1991b; Ruwald et al. 1991) and banded morwong *Cheilodactylus spectabilis*, (Searle and Zacharin 1994). In the tropics, most research effort has been expended on barramundi. However, research into new species is focusing on coral trout *Plectropomus leopardus*, golden snapper *Lutjanus johnii*, and mangrove jack *Lutjanus argentimaculatus* (Rimmer et al. 1994a).

In temperate waters snapper *Pagrus auratus*, mulloway *Argyrosomus hololepidotus*, sand whiting *Sillago ciliata*, and mahi mahi *Coryphaena hippurus* were seen as good farming candidates (Anon 1991a; Battaglene and Bell 1991; Young 1991; Treadwell et al. 1992). Both snapper and mulloway may also be suitable for stock enhancement, based on successful enhancement programs with the same or similar species overseas (Foscarini 1988; Battaglene and Bell 1991; Ungson et al. 1993, 1994).

TABLE 1 Cultured Australian marine fin-fish produced, or under investigation for, commercial farming or enhancement programs.

COMMON NAME	SCIENTIFIC NAME	CLIMATE	STATE OR TERRITORY
Atlantic salmon [a]	*Salmo salar*	Coldwater	TAS
Sea trout[a]	*Oncorhynchus mykiss*	Coldwater	TAS
Greenback flounder[b]	*Rhombosolea tapirina*	Coldwater	TAS
Long-snout flounder	*Ammotretis rostratus*	Coldwater	TAS
Striped trumpeter	*Latris lineata*	Coldwater	TAS
Banded morwong	*Cheilodactylus spectabilis*	Coldwater	TAS
Black bream	*Acanthopagrus butcheri*	Temp/Cold	TAS & WA
Yellowfin bream	*Acanthopagrus australis*	Temperate	QLD
Southern blue-fin tuna[a]	*Thunnus maccoyii*	Temperate	SA
Australian bass [a]	*Macquaria novemaculeata*	Temperate	NSW and QLD
Snapper[b]	*Pagrus auratus*	Temperate	NSW, SA & WA
Mulloway[b]	*Argyrosomus hololepidotus*	Temperate	NSW & SA
Sand whiting	*Sillago ciliata*	Temperate	NSW & QLD
Trumpeter whiting	*Sillago maculeata*	Temperate	NSW
WA Jewfish	*Glaucosoma hebraicum*	Temperate	WA
Dolphin fish	*Coryphaena hippurus*	Trop/Temp	QLD
Barramundi[a]	*Lates calcarifer*	Tropical	QLD, SA & NSW
Coral trout	*Plectropomus leopardus*	Tropical	QLD
Golden snapper	*Lutjanus johnii*	Tropical	NT & QLD
Mangrove jack	*Lutjanus argentimaculatus*	Tropical	QLD
Estuary cod	*Epinephelus tauvina*	Tropical	QLD

[a] Produced commercially

[b] Experimental growout

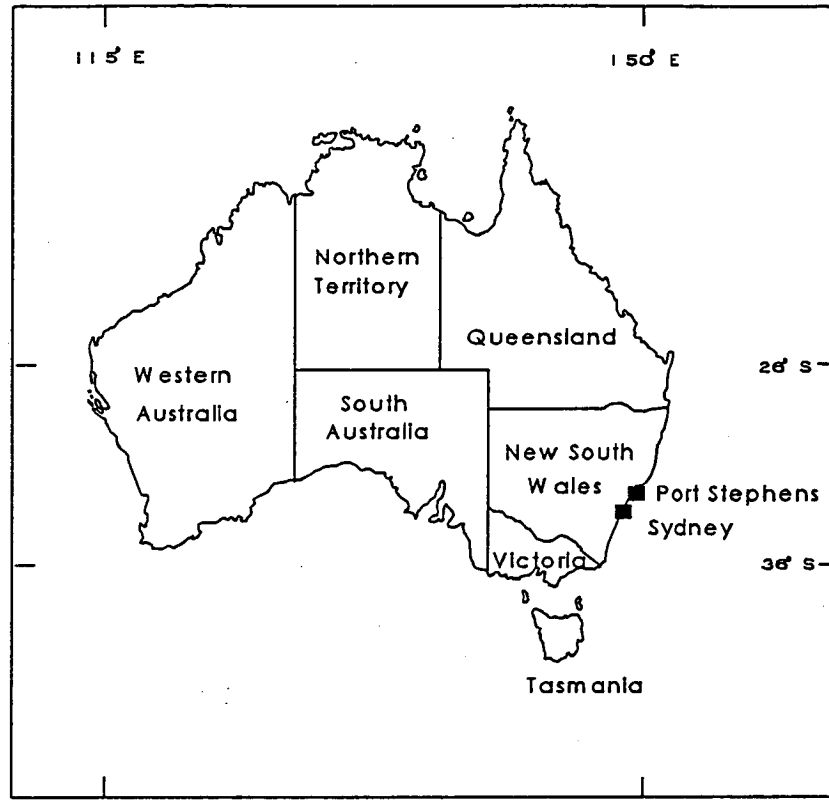


Figure 1 Map of Australia showing the location of the Port Stephens Research Centre and the Fisheries Research Institute in Sydney.

4. SPECIES STUDIED FOR THIS THESIS

The hormone-induction and larval rearing of four species of Australian temperate marine fishes were examined for this thesis. These species were: Australian bass *Macquaria novemaculeata* (Steindachner 1866), family Percichthyidae; snapper *Pagrus auratus* (Bloch and Schneider, 1801), family Sparidae; sand whiting *Sillago ciliata* Cuvier 1829, family Silliganidae and mulloway *Argyrosomus hololepidotus* (Lacepede 1801), family Sciaenidae . All are recreationally and/or commercially important species in New South Wales.

Australian bass is a native catadromous species found in the coastal drainages of southeastern Australia (MacDonald 1978). It is one of Australia's most highly prized sport fish and is endemic to the coastal streams of eastern Australia. Commercial marine larviculture in Australia using modern live food techniques started with *M. novemaculeata* in New South Wales in 1979. *Macquaria novemaculeata* were bred by NSW Fisheries to stock public impoundments to provide a resource for recreational anglers. In comparison to many new aquaculture candidates, its biology is well understood (Williams 1970; Schnierer 1982; Harris 1983,1985,1987,1988; Langdon 1987). Early breeding research has been reviewed by Battaglene et al. (1989a). Initial research concentrated on determining the effects of temperature, salinity and concentration of live food on hatch and survival of eggs and yolk-sac larvae (van der Wal 1985; van der Wal and Nell 1986).

Culture of *M. novemaculeata* on a commercial scale has been hampered by the absence of functional swim bladders in intensively reared larvae, a nutritionally based metabolic disorder, and difficulties in acclimating larvae to fresh water (Battaglene et al. 1989b). Several hatcheries in New South Wales and southern Queensland now produce some *M. novemaculeata* primarily for stocking farm dams and freshwater impoundments for recreational sport fishing (Battaglene and Allan 1990). There is no commercial harvest of wild *M. novemaculeata*.

Macquaria novemaculeata is useful as a model experimental animal to help develop larval rearing protocols that can be adapted for other more commercially selected species. It is an ideal species for this purpose because wild and captive broodstocks are readily available, preliminary studies have been done to detect problem areas in larval rearing, and it breeds in winter which is out -of-season with other targeted species. In addition, it is a difficult species to breed and presents all of the common problems associated with marine larviculture. Finally, there are many similarities in the biology, breeding and larval rearing requirements of *M. novemaculeata* and striped bass *Morone saxatilis* (Harrell et al. 1990) and European sea bass *Dicentrarchus labrax* (Barnabe 1988).

Snapper is a very important commercial and recreational species in Australasia (Bell et al. 1991; Francis 1994). The biology of *P. auratus* is well understood in New Zealand (eg. Scott

and Pankhurst 1992; Scott et al. 1993; Francis 1994) and Japan (Foscarini 1988; Fukusho 1989) but not Australia (Henry 1988). *Pagrus auratus* was selected as the species with the most immediate potential for marine farming in New South Wales because it is well known and regarded, has a good established domestic market profile, wild stocks are declining, and an export market exists in Asia for farmed fish (Battaglene and Bell 1991). The total annual catch of *P. auratus* in Australia is about 2000 t. In New South Wales catches have declined from 1000 t in 1980 to 440 t in 1990. In 1991-92 *P. auratus* prices at the Sydney Fish Markets averaged AUD$7.76/Kg (McNee et al. 1993).

Pagrus auratus was formerly named *Chrysophrys auratus* in Australia and New Zealand, and *Pagrus major* (red sea bream) in Asia. It is now recognised as a single species with independent and reproductively isolated populations in Japan and Australasia (Paulin 1990). However, there are taxonomists who are not yet convinced these populations are conspecific (Taniguchi et al. 1986; J. Paxton personal communication, 1994). Irrespective of these concerns, the aquaculture production of red sea bream in Japan (Foscarini 1988; Fukusho 1989 1991) and the Mediterranean (Sweetman 1992) provides a firm technical basis for the development of *P. auratus* farming in Australia. Additional information is also available on the culture of other sparids, for example, gilthead sea bream *Sparus auratus* in France (Barnabe 1988).

Pagrus auratus was first artificially bred in Japan in 1887, although commercial farming did not start till the 1960's (Davy 1990). In contrast, *P. auratus* was first bred in New Zealand (Pankhurst and Pankhurst 1989; Pankhurst et al.1991) and Australia (Evans 1989) in the late 1980's. Government research hatcheries producing relatively small numbers of juvenile *P. auratus* are now established in New South Wales and Western Australia. Experimental small-scale sea cage farming of *P. auratus* started in New South Wales in 1992 and Western Australia in 1994.

Sand whiting is commonly found in northern New South Wales and southern Queensland (McKay 1985). The total annual catch of whiting (including *S. maculeata*) has recently increased to 1150 t in 1990 but only 200 t was caught in New South Wales. This species was chosen because it is euryhaline, breeds in captivity and can be hormone-induced to spawn (Goodall et al.1987; Young 1991). It is a highly regarded species with a good market profile consistently fetching high prices. In 1991-92 the average wholesale price at the Sydney Fish Market was AUD$6.68/ Kg (Kailola 1993).

The biology of *S. ciliata* in the wild is relatively well understood (Burchmore et al. 1988). They are found in estuaries and near shore waters, are multiple spawners and benthic feeders reaching a maximum size of around 1.5 Kg (Kailola 1993). The aquaculture potential of *S. ciliata* was first investigated in the late 1980's in Queensland. This research

concentrated on sperm preservation, hormone-induced ovulation and egg and early larval development (Young 1991). However, larvae were not reared past yolk-sac absorption (Young 1991). In Japan, *S. japonica* has been intensively cultivated and is often used in early life stage experiments (Khan et al. 1991; Oozeki et al. 1992).

Mulloway has an Indo-Pacific distribution and is an important commercial and recreational species in Australia and southern Africa. The total annual catch of *A. hololepidotus* in Australia between 1964 and 1990 has varied from 550 to 225 t . In New South Wales catches have declined from 450 t in 1974 to 160 t in 1990. In 1991-92 the average wholesale price at the Sydney Fish Market was AUD$5.85/ Kg (Kailola et al. 1993).

Argyrosomus hololepidotus was selected because it is widely distributed, has a good domestic market profile, is highly fecund, euryhaline, and grows quickly in captivity (Battaglene and Bell 1991; Gray and McDonall 1993). Unlike the other species investigated, little information was available on the biology of *A. hololepidotus* and it had not been bred in captivity before. Several other sciaenids are cultivated overseas including red drum *Sciaenops ocellatus*, orangemouth corvina *Cynoscion xanthulus* and white seabass *Atractoscion nobilis* (Orhun 1989; Holt et al. 1990).

5. PROVISION OF SEED STOCK

All aquaculture operations are dependent, to some level, on the reliable supply of fertilised eggs, or juveniles (Pankhurst and Pankhurst 1989). Usually, it is not practical to base an aquaculture industry on the collection of wild-caught seed. Notable exceptions do exist, for example, yellowtail *Seriola quinqueriata* in Japan (Davy 1990), and tuna farming in South Australia (Nicoll 1993). However, in most cases appropriate techniques for the controlled reproduction and larviculture of new species need to be developed (Shelton 1989; Sorgeloos and Leger 1992).

The production and rearing of marine fish larvae usually involves collection of broodstock; hormone induction; incubation of eggs and yolk-sac larvae; feeding of live food to larvae; and final weaning of juveniles onto artificial foods. There are currently two problem areas in the provision of seed stock in Australia. First, difficulties have been experienced ensuring a reliable supply of captive or wild-caught broodstock and consequently good quality eggs (Treadwell et al. 1992). Second, the conditions necessary for successful larval rearing of most species have not been determined and many species have not been reared through to metamorphosis. Consequently the larval rearing stage has been a major "bottleneck" in the production of marine fin-fish.

Overcoming larviculture problems took up to 20 years of research and development before commercial production of some species began in Europe (Stanley 1991; Treadwell et al.

1992). Although the time required to develop culture techniques for new aquaculture species in Australia could now be expected to be shorter, the transfer of technology between countries and species is not always appropriate or trouble free (Stanley 1991; Treadwell et al. 1992).

5.1 Reproduction and Hormone Induction

Control over reproduction is essential to success in aquaculture (Shelton 1989). Eggs can be obtained from: plankton collection from the wild; spontaneous spawning or stripping of ovulating eggs from wild-caught adults; stripping or spontaneous spawning of wild or captive adults in which ovulation has been induced by hormone injection; spontaneous spawning of captive adults with or without environmental manipulation (Hunter 1984; Shelton 1989; Tucker and Jory 1991).

Most research programs studying new species begin by obtaining eggs from wild fish, particularly if they can be readily caught in mature condition. However, the use of captive broodstock has many advantages. Some species are difficult to catch in spawning condition and successful spawning, even with hormone treatment, can be impaired by the stress of capture (Pankhurst and Sharples 1992). Captive broodstock can also often be manipulated to spawn over longer periods than wild fish (Lam 1983). Furthermore, domesticated broodstock, ie broodstock from successive generations of hatchery fish are usually easier to spawn, and can produce better quality eggs (Foscarini 1988). In Australia this "closing of the life cycle" and year-round spawning in marine fish has only been achieved with *L. calcarifer* and mahi mahi *Coryphaena hippurus* (Garrett and Rasmussen 1987; Anon 1991b; Anderson et al. 1993).

The ultimate aim of most fish hatcheries is to induce spawning in captivity usually through the manipulation of environmental parameters such as photoperiod, temperature, and salinity (Lam 1983; Shelton 1989). In general, long or increasing photoperiod and/or high or rising temperature stimulates gametogenesis in spring and summer spawners whereas the reverse conditions simulate autumn or winter spawners (Lam 1983). Special care needs to be given to ensure captive broodstock are not stressed by inappropriate manipulation of environmental parameters, disease, and human disturbance (Sumpter et al. 1987). In addition, the diet of broodstock is an important factor determining both the quantity and quality of eggs produced from captive broodstock (Watanabe et al. 1984a,b).

Many fish may complete gametogenesis but fail to spawn in captivity (Pankhurst and Pankhurst 1989; Zohar 1988). Hormones (and other compounds) are often used to induce final oocyte maturation, ovulation, courtship behaviour and spawning (Donaldson and Hunter 1983). Major reviews of teleost endocrinology and the use of hormones in fish culture have been published by Goetz (1983), Donaldson and Hunter (1983), Zohar (1988) and Shelton

(1989). The commonly used hormones include: fish pituitary extracts; fish gonadotropin (GtH); mammalian gonadotropin (eg. human chorionic gonadotropin, hCG; luteinising hormone, LH); gonadotropin releasing hormones (GnRH) and analogs (eg. luteinising hormone releasing hormone analog, LHRHa); maturation-inducing steroids (MIS); prostaglandins; antiestrogens; catecholamines. Hormones, particularly hCG and LHRHa, are commonly used to induce spawning in Australian native freshwater fish (eg. Rowland 1983, 1984, 1988; Ingram and Rimmer 1992), catadromous species (Garrett and Rasmussen 1987; Battaglene et al. 1989a) and some marine fish (Crawford 1984,1986; Young 1991).

For each new species, the following aspects of hormone induction and conditions necessary for successful production of fertilised eggs need to be determined: choice of hormone, hormone dose, method and timing of administration. Similarly, the determination of the time between hormone administration and ovulation (latent period), and the need for intervention and hand stripping of gametes varies between species. Successful hormone-induced ovulation, fertilisation and egg incubation requires an understanding of the breeding season, size and age at maturity, type of spawning (eg. synchronous or asynchronous), fecundity and the nature of eggs produced (eg. pelagic or demersal). Selection of spawning tanks, spawning protocols and control of environmental parameters particularly temperature is also species-specific. These factors can also vary for different sources of broodstock (captive versus wild) collected at different times during the breeding season. The large number of procedural variations possible means that no single study or series of studies has investigated all the relevant variables in a single species (Donaldson and Hunter 1983).

5.2 Larviculture

A good understanding of the biology and ecology of selected species can make the culture of marine fish more economical by limiting the naturally high larval mortality (Baxter 1988; Foscarini 1988). The more important developmental and behavioural traits influencing larval survival in intensive culture systems are shown in Figure 2. These are factors used in the selection of new species. Ideally all factors influencing survival should be experimentally tested to find optimal rearing protocols. In practise this level of sophistication has rarely been achieved (Shepherd and Bromage 1988).

The interactions among the biotic and abiotic factors influencing larval survival also need to be considered in the determination of optimal rearing conditions (Fig. 2). Larval density and temperature are particularly important factors because they can influence larval survival, development and growth. In general, initial larval rearing trials with new species should be conducted at low densities within the environmental range of the species in the wild. This provides the best chance of rearing larvae through to metamorphosis and establishing 'normal' development and growth rates. Success with rearing new species will depend on: a

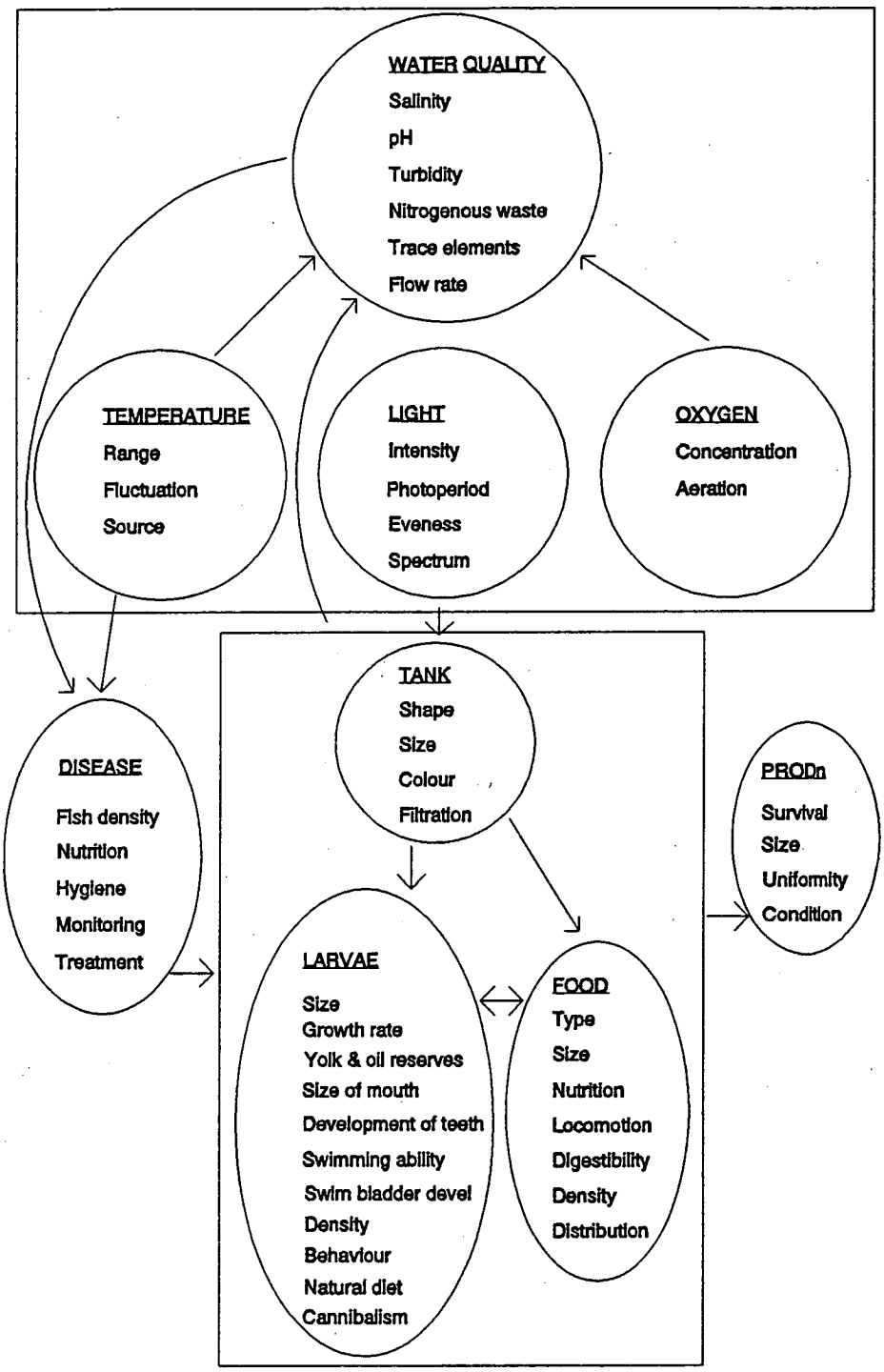


Figure 2 The relationship of major categories of factors influencing the survival and growth of intensively reared marine fish larvae

reliable supply of eggs; the development and behaviour of the larvae (ie intrinsic biology); the facilities available; and the experience of the researchers.

Although, there has been considerable progress and improvement in larviculture over the past decade, especially in larval nutrition, technical methods and disease diagnosis and control, the determination of optimal larval rearing conditions needs to be undertaken for each new aquaculture species (Hunter 1984; Blaxter 1988; Shepherd and Bromage 1988; Sorgeloos and Leger 1992). Clearly, it is faster and easier to establish hatchery protocols for species that are taxonomically similar to other cultivated species. Two relevant species for which rearing protocols have been developed overseas are: the European sea bass *Dicentrarchus labrax*, family Moronidae; and gilthead sea bream *Sparus auratus*, family Sparidae (Barnabe 1988).

5.3 Initial Swim Bladder Inflation in Fish Larvae

Larval mortality often peaks during or soon after the transition from endogenous to exogenous feeding (May 1974). At first feeding, larvae typically have poorly developed eyes, digestive systems and swimming abilities (Blaxter 1988). Initial swim bladder inflation also occurs in many species about this time. In the majority of teleost fish, the swim bladder is a hydrostatic organ which regulates buoyancy. Larvae with dysfunctional swim bladders need to expend more energy swimming and may find it more difficult to feed. The swim bladder can also play an important role in perception, sound production and respiration (Steen 1970). The structure and function of the swim bladder in adult fish are well known (see reviews by Harden-Jones 1957; Steen 1970; Fange 1983). However, little is understood about the timing and mechanisms of initial inflation in most species (Chatain 1989).

Failure of the swim bladder to inflate has been associated with mass mortality in marine fish during intensive culture (Spectorova and Doroshev 1976), but more commonly it results in reduced growth and increased susceptibility to stress-induced mortality (Al-Abdul-Elah et al. 1983a,b; Chatain 1987; Chapman et al. 1988). Fish without functional swim bladders can also develop severe skeletal deformities (Paperna 1978; Kitajima et al.1981; Weppe and Bonami 1983; Anon 1987; Chatain 1994). Hypertrophy of the swim bladder can also occur , usually some weeks after the start of exogenous feeding, and is not associated with initial inflation (Nash et al. 1977; Johnson and Katavic 1984; Bagarino and Kungvankij 1986; Katavic 1986).

Factors influencing swim bladder inflation vary between species and include egg quality, water quality, tank hydrodynamics, salinity, temperature, and food quality (Hadley et al. 1987; Chatain and Ounais-Guschemann 1990). The elimination of oily surface films can greatly increase swim bladder inflation rates in intensively cultured larvae that gulp air on the water

surface (Chatain and Ounais-Guschemann 1990). For many species the conditions for inflation have not been determined and even under so called 'optimal conditions' many larvae may still fail to inflate their swim bladders (Hadley et al. 1987; Chatain and Ounais-Guschemann 1990).

6. PURPOSE OF THIS THESIS

The overall goal of research described in this thesis was to develop techniques for the large-scale production of juvenile marine fish in New South Wales. Specifically the aims were to induce ovulation in broodstocks and rear to metamorphosis the larvae of *M. novemaculeata*, *P. auratus S. ciliata* and *A. hololepidotus*.

The major objectives for each species were:

1. To determine when fish matured in the wild
2. To develop collection and transportation techniques
3. To examine the effects of hormone treatments on ovulation and spawning in wild-caught and captive fish
4. To determine the latent period, number of eggs, fertilisation and hatch rates for fish induced to ovulate using hormones
5. To describe the development and growth of larvae reared intensively on rotifers and brine shrimp
6. To determine the timing of, and factors influencing initial swim bladder inflation in intensively cultured larvae
7. To compare and contrast the relative difficulties of producing the four species and suggest where future research should be directed

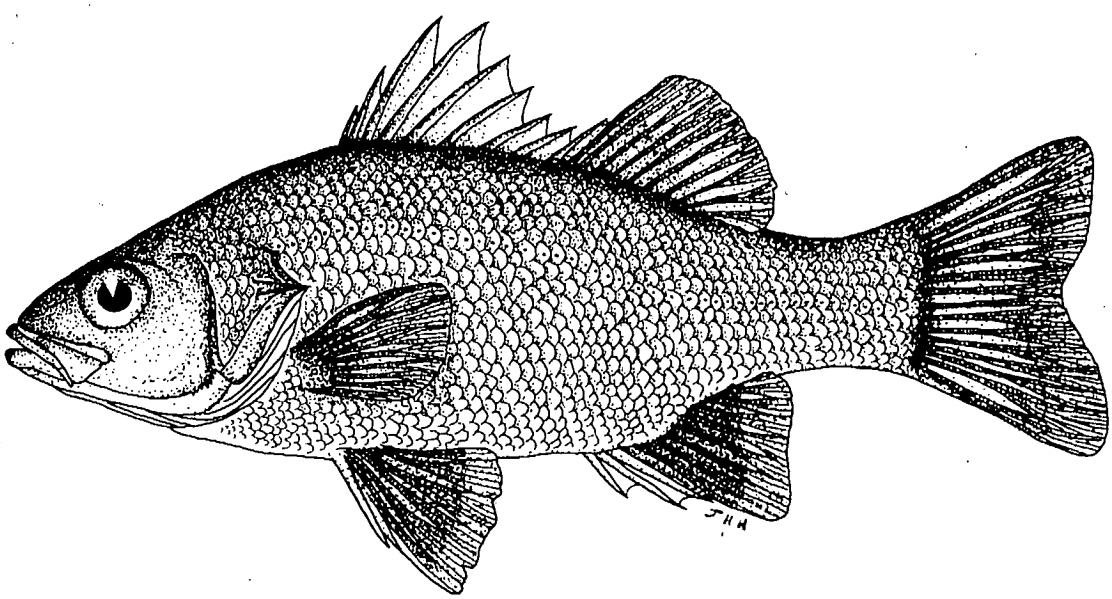
The thesis is presented as a series of papers referred to in the text as Chapters 2 to 11. As a result there is some repetition in the Introduction, Discussion and Reference sections of some chapters. References for Chapter 1 and 12 are given at the end of the thesis. Chapter 1 is a general introduction and provides information on the status of fish farming and larviculture in Australia. This chapter also outlines the aims and objectives of the thesis. The next four chapters (2 to 5) relate to *M. novemaculeata*. Chapter 2 examines eight years of hatchery data on the optimum hormone doses for ovulation and spawning in wild and captive *M. novemaculeata*. Chapters 3 and 4 describe laboratory experiments on larval development and factors influencing initial swim bladder inflation. The intention was to detect the most important factors in initial swim bladder inflation. Factors tested included light intensity, salinity, and aeration. Chapter 5 provides a summary of 20 trials in saltwater ponds aimed at increasing the survival and growth of cultured larvae. The chapter examines the effects of temperature and food resources on growth and survival of larvae through to metamorphosis.

Chapters 6 to 8 report the research into the induced spawning and larval rearing of *P. auratus*. Chapter 6 is the first published account of *P. auratus* breeding in Australia and provides a comparison of the development of *P. auratus* larvae reared in Japan, New Zealand and Australia. Chapter 7 provides a summary of early rearing trials and outlines major problems in spawning induction of wild fish. Chapter 8 examines the induced spawning of both wild and captive *P. auratus* using a range of hormone types.

Chapters 9 and 10 report research into the hormone induction and larval rearing of *S. ciliata*. Chapter 9 examines the hormone induction of wild fish. Chapter 10 describes for the first time the larval rearing of *S. ciliata* through to metamorphosis. In addition, it also presents laboratory experiments to determine larval development and the effect of light and surface access on swim bladder inflation. Chapter 11 provides the first published account of the induced spawning and larval rearing of *A. hololepidotus*, including a comparison of the larval development of other cultured sciaenids.

In Chapter 12, the general discussion, the research results are discussed in relation to the development of marine larviculture in Australia and the rest of the world. Particular emphasis is placed on the problem of initial swim bladder inflation in cultured larvae and how the results presented in this thesis increase our understanding of, and provide new ways to overcome this major world-wide problem. Throughout Chapter 12 the relative advantages and disadvantages of the four species for mass seed production are discussed and recommendations made regarding future research directions.

PLATE 1 Australian bass, *Macquaria novemaculeata*
(Drawn by John Harris)



CHAPTER 2

Hormone-induced ovulation and spawning of captive and wild broodfish of the catadromous Australian bass *Macquaria novemaculeata* (Percichthyidae)

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Accepted for Publication in

AQUACULTURE AND FISHERIES MANAGEMENT

ABSTRACT

Australian bass, *Macquaria novemaculeata*, mature but do not spawn in fresh or brackish water ponds. Ovulation and spawning of captive (n=158) and wild Australian bass (n=123) was induced in the normal breeding season by single injections of human chorionic gonadotropin (hCG). Doses of 100 to 4000 I.U.kg^{-1} hCG induced ovulation and the optimum dosage was 500 I.U.kg^{-1} hCG. The breeding season was from mid May to late August for wild fish and extended into September for captive fish. There was a tendency for mean fertilisation and hatching success to decline over the breeding season. Higher fertilisation and hatching success were obtained from fish which spawned naturally than from stripped fish. Fish spawned after 34.2±0.4 h (mean±SE, n= 74). Ovulating fish that failed to spawn were stripped after 40.2±0.3 h (n=76). The timing of stripping and fertilisation was an important factor determining hatching success. There was no apparent difference in latency periods, or the number of eggs spawned, between captive and wild fish. However, the mean number of eggs obtained from naturally spawned fish was higher than for stripped fish. The techniques described in this paper will assist the large-scale production of Australian bass by increasing the quality and quantity of larvae from hCG-induced spawnings.

INTRODUCTION

The Australian bass, *Macquaria novemaculeata* (Steindachner), is a catadromous percichthyid fish native to the coastal drainages of south-eastern Australia (MacDonald 1978) migrating downstream in winter to spawn in brackish water. It is a long-lived and slow growing species and a highly fecund serial spawner (Harris 1985, 1986). The species is also a highly-prized sport fish but has declined in distribution and abundance partly because of dam and weir construction, habitat degradation and overexploitation by recreational and commercial fishing (Harris 1988; Battaglene, Beevers & Talbot, 1989a). There is consequently a large public demand for the fish to stock farm dams, lakes and impoundments (Battaglene et al. 1989a).

In the wild, the later stages of gonadal development, and the migration of female Australian bass from freshwater to the brackish spawning zone are dependent on flooding (Harris 1986). The closely related golden perch *Macquaria ambigua,* can be artificially induced to spawn in ponds by temperature and water level manipulation (Lake 1967). However, the physiochemical conditions that exist in flooding rivers are difficult to reproduce and *Macquaria novemaculeata* have not been successfully spawned in captivity without the use of hormones (van der Wal, unpublished data 1985; Shelton 1989; Battaglene et al. 1989a).

The Port Stephens Research Centre, in New South Wales (NSW) (32° 45'S, 152° 04'E) was the first hatchery to develop and apply mass-propagation techniques to *M. novemaculeata* (van der Wal 1985; Battaglene et al. 1989a). The commercial production of *M. novemaculeata* juveniles is now established in southern Queensland and NSW using the techniques described by Battaglene, Talbot & Beevers (1989b). Hatchery production of Australian bass still depends on the capture of mature broodfish from local farm dams or rivers during the breeding season. The capture of wild fish is unpredictable, highly seasonal, and tightly controlled to protect declining stocks. Captive broodfish do not spawn in captivity; however, the induction of final oocyte maturation, ovulation and spawning in Australian bass is possible with human chorionic gonadotropin (hCG) (Battaglene et al. 1989a).

Responses to exogenous gonadotropins are species-specific and although optimum dosages and latency periods have been established for other Australian percichthyids (Rowland 1983, 1988; Gooley & McDonald 1988; Ingram & Rimmer 1992) very little empirical data exists for *M. novemaculeata*. Optimising hormone dosages reduces hatchery costs and increases the quality and quantity of eggs produced. Improved techniques also reduce the number of wild fish collected for broodstock.

The aims of the current study were to determine 1) the optimum dosage of hCG for ovulation and spawning 2) the latency period for hormone induced wild and captive broodfish to ovulate and 3) to determine if differences existed in the number of fish which spawned and the number of eggs produced in wild and captive broodfish injected with hCG.

MATERIALS & METHODS

This study was undertaken at the Fisheries Research Centre, Port Stephens NSW, over nine consecutive breeding seasons starting in July 1984. The data are divided into trials with captive fish (1984-1986 and 1990) and wild fish (1987-1992).

Capture and maintenance of broodfish

Experiments with captive *M. novemaculeata* refer to fish collected from the wild and held in ponds. Adult Australian bass were captured with gill nets and held for at least six months in 100 000 l concrete-lined ponds in brackish water (2-15 ‰) prior to experimentation. The fish were held at densities of approximately 40 fish $pond^{-1}$ (95 m^2) and fed on prawns, mullet and artificial pellets (Battaglene et al. 1989a). Mean monthly pond temperatures during the breeding season (June-September) varied from 10 to 22°C (Maguire & Bell 1982).

Wild fish trials used Australian bass collected with gill nets above the Williams River weir at Seaham (fresh water), NSW. Fish were collected in the morning and held in an aerated 750 l transporter, in fresh water, for a maximum of 8 h. Fish were transferred (without salinity acclimation) to 350 l tanks supplied with sea water (30 to 35 ‰) at ambient temperature (13-18 °C) for 1-2 h before being used in experimental protocols.

Broodfish handling and sampling of oocytes and milt

Prior to examination, captive fish were anaesthetised with quinaldine (60 mg/l) or ethyl ρ-aminobenzoate (30 mg/l). The techniques for the sampling of oocytes and milt, and the injection of hormones were as described by Battaglene et al. (1989a).

Oogenesis was divided into four stages (see Battaglene et al. 1989a; Harris 1985). Stage I comprised oogonia and primary oocytes, stage II yolk vesicle and yolk globule oocytes, stage III yolk mass oocytes and stage IV atretic oocytes. Females were considered ready for spawning when the majority of ova were stage II or III (Battaglene et al. 1989a) . From 1988 onwards the ten largest oocytes were measured (±30μm) for each fish and the number of oocytes at each stage estimated to the nearest 5 %.

Spermiated males were used in all trials. Sperm collected from each male was diluted with sea water and examined microscopically (x 400). Only males with very active sperm (Harris 1986) were used. One male and one female were placed in each spawning tank following hormone treatment. Spawning tanks (2,000 l till 1985; 750 l after 1986), had no water exchange but were well aerated to keep the eggs in suspension. The fish in early trials (1984-6) were subjected to natural photoperiod while those in later trials were kept in the dark. Water temperatures were maintained at 18±1°C and salinity at 25 to 35‰. These conditions are considered within the optimum range for Australian bass eggs and yolk-sac larvae (van der Wal 1985).

Injection procedure and hormone dosages

The preparation and administration of hormone dosages followed Rowland (1983). HCG (Pregnyl ®, Organon Australia Pty Ltd) was dissolved in distilled water (1 ml kg^{-1}) immediately prior to injection and was administered per unit body weight by intraperitoneal injection at the posterior base of the left pelvic fin. Females in control treatments were injected with 1 ml kg^{-1} of distilled water. Males were injected with 200 I.U.kg^{-1} of hCG. All fish were injected between 16.00 and 19.00 h on the day of capture.

Experiment 1: Effect of hCG dose on captive fish

The effect of hCG on ovulation, spawning, fertilisation and hatching success of captive Australian bass was examined in 1984-1986 using hormone doses ranging from 0 to 4000 I.U.kg^{-1}. Data for 101 fish injected with 500, 1000 or 2000 U kg^{-1}, in June, July and August (Table 1) were statistically compared. Nine fish caught in September were excluded because they had started atresia. Data from 10 fish injected with 0, 100 or 4000 I.U.kg^{-1} were not statistically analysed because of the small sample sizes.

Experiment 2: Effect of low hCG dose on captive fish

An additional hormone induction trial with captive females was conducted in June 1990 to determine the effect of lower hCG doses. In this trial, 16 pairs of fish (one male and one female) from one pond were allocated to separate spawning tanks and females were injected with one of four hCG doses (0,100,200 or 500 I.U.kg^{-1}). Four replicate females were randomly chosen for each dose.

Experiments 3 and 4: The effect of hCG dose on wild fish

Wild females were injected with 0, 500 or 1000 I.U.kg^{-1} hCG. A total of 123 wild females were injected (Table 2). Fish caught in 1988 and 1989 were injected with 1000 I.U.kg^{-1} hCG and were used to determine seasonal changes in fertilisation and hatch rates (Experiment 3). Fish caught in June and July of 1990, 1991 and 1992 were injected with 500 I.U.kg^{-1} hCG (Experiment 4).

Experiment 5: Hormone induction of females without males

Nine wild-caught females were injected with 500 I.U.kg^{-1} hCG and placed in tanks without males to determine if females required the presence of males to ovulate and whether they could be successfully stripped.

Determination of ovulation and spawning

Latency period is defined as the time between injection and ovulation. In the early dose trials (1984-86) the spawning tanks were dip netted every 30 minutes starting 24 hours after injection to assess if and when fish had spawned. Females which had not spawned after 38 h were anaesthetised and ovulating fish were stripped. If ovulation had not started the female

was returned and inspected approximately one hour later.

During wild fish trials the time of spawning was estimated from the number of cell divisions in fertilised eggs collected after 38 h. The development time for the early cell divisions at 18°C was calculated as 1 h for the first division, 45 min for the second, and 30 min for each subsequent division.

Determination of egg production, fertilisation and hatching success

The percentage of fertilised eggs was determined by microscopically examining 200 eggs . Water-hardened eggs were measured volumetrically and incubated according to Battaglene et al. (1989a). The water temperature in incubators was maintained at 19±1°C. Two hours before hatching 200 eggs from each aquaria were sampled and hatching success calculated from the number of active embryos counted.

Statistical analysis

Fertilisation and hatch data, expressed as proportions, covered a wide range of values including 100%, and were arcsin transformed to meet the assumptions of normality and homogeneity of variance (Steel & Torrie 1960). Homogeneity of variances was evaluated using Bartlett's test (Steel & Torrie 1960). The effect of hormone dose on fertilisation and hatch were examined separately for fish which spawned and for those stripped by one-way ANOVA. Values are given as mean±SE.

RESULTS

Australian bass matured but did not spawn in brackish water ponds. Females had distended abdomens and inflamed vents during the breeding season. Males were spermiating from April to September. None of the wild or captive females in control treatments (n=15) ovulated or spawned. Successful hormone-induced spawning using wild fish occurred from mid May to late August. One wild female did spawn in April 1988, but the eggs (96% fertilisation) failed to hatch. Captive Australian bass were successfully induced to spawn from early June to mid September. Of the 281 females injected only two captive fish and four wild fish died.

The oocyte stages for both captive and wild fish showed a progression from predominantly stage II eggs in June to stage III in August. The progression was more pronounced in wild fish (Table 3). Ovarian samples taken from fish with stage II oocytes had mean diameters of 0.86±0.01mm (mean±SE, n=52), those with stage III oocytes, 0.90±0.01mm (n=9). Australian bass with predominantly stage II and or stage III eggs were successfully induced to ovulate and spawn. Females with only stage I eggs (mean diameter 0.52±0.04, n=7) or those with a majority of stage IV eggs prior to injection had low hatch rates. For example, the hatch rates obtained from wild fish injected with 1000 I.U.kg-1 hCG with mainly stage I eggs (n=7) was zero, stage II (n=26) 65.4%, stage III (n=10) 40.4% and stage IV (n=2) zero.

Experiment 1: Effect of hCG dose on captive fish

For captive fish injected with doses of 500 I.U.kg^{-1} or above 60% spawned, 19% were successfully stripped and 21% failed to ovulate (Table 4). There was no significant difference in mean fertilisation (df=45, F=0.134, P>0.05) or mean hatch (df=36, F=2.46, P>0.05) between the dosages for which fish spawned or were stripped (mean fertilisation df=14, F=0.234, P>0.05; mean hatch df=13, F=2.552, P>0.05) (Table 4). At 1000 and 2000 I.U.kg^{-1} hCG higher fertilisation and hatching success were obtained from eggs from fish which spawned than from fish which were stripped (Table 4). There was also a tendency for mean fertilisation and hatch rates to decline over the breeding season particularly in fish injected in September (Fig. 1). However, it was difficult to separate variation between months from the large variation in the response exhibited at each dosage (Table 4).

Experiment 2: Effect of low hCG dose on captive fish

Dosages as low as 100 I.U.kg^{-1} induced ovulation and spawning in captive fish but at this dose fertilisation and hatching success were very low (Table 5). Similarly, a dose of 200 I.U.kg^{-1} induced spawning but led to significantly lower and more variable fertilisation and hatching (Table 5). All females injected with 500 I.U.kg^{-1} spawned and had fertilisation rates >80%.

Experiments 3 and 4: The effect of hCG dose on wild fish

From 1988 to 1992 a total of 52 female Australian bass were injected with 1000 I.U.kg^{-1} hCG and 34 with 500 I.U.kg^{-1} hCG. Overall for 1000 I.U.kg^{-1} hCG, 35% of females spawned, 28% were stripped and 37% failed to ovulate (Table 6). For 500 I.U.kg^{-1} hCG, 62% spawned, 29% were stripped and 9% failed to ovulate (Table 7).

Mean fertilisation and hatching success for fish which spawned after injection with 1000 I.U.kg^{-1} hCG were 96.2±1.0% and 77.7±8.3% respectively. These values were higher than the corresponding values for stripped fish which were 71.7±6.3% and 69.2±9.5% respectively (Table 6). Similar fertilisation and hatching success to those achieved with 1000 I.U.kg^{-1} hCG were obtained with 500 I.U.kg^{-1} hCG for both spawned and stripped fish (Table 7).

Experiment 5: Females without males

All females given 500 I.U.kg^{-1} hCG in the absence of males (n=9) ovulated and seven were successfully stripped and fertilised (Table 7). The other two fish released eggs in the spawning tank prior to the 38 h ovulation check. Sixty two percent of the females in the presence of males (n=21) spawned, 29% were successfully stripped (n=10) and 9% failed to ovulate (n=3) (Table 7).

Number of eggs from captive and wild females

The relationship between the weight of wild and captive females injected with 500 or 1 000 I.U.kg^{-1} hCG and the number of eggs from spawned and stripped fish is given in Figs. 2 and 3. In general, fish which spawned produced more eggs kg^{-1} for both wild and captive females than stripped fish. Post-spawning females after dissection had flaccid gonads with a few remnant ripe eggs. Stripped females after dissection occasionally retained vitellogenic oocytes in the process of clearing.

Time between injection and spawning or stripping

There was no apparent difference in the time taken between injection and spawning for captive and wild fish injected with doses of hCG from 500 to 2000 I.U.kg^{-1} (Fig. 4). The mean time taken for captive and wild fish to spawn following induction with 500, 1000 and 2000 I.U.kg^{-1} was 34.2±0.4 h, n=74.

Similarly, there was no apparent difference in the time between injection and stripping for captive and wild fish (Fig. 4). The mean time for stripped fish was 40.2±0.3 h, n=76. Seventy percent of stripped fish treated with 500 or 1000 I.U.kg^{-1} hCG (n=64) were successfully stripped between 38 and 41 h after injection (Fig. 5). There was a reduction in hatching success after 41 h for both stripped (Fig. 5) and spawned fish.

DISCUSSION

HCG is currently the most common gonadotropin used to induce ovulation in fish because it is highly effective for many species, is widely available, has excellent storage characteristics and standardised dosage forms (Rowland 1983; Shelton 1989). Optimum dosages of hCG, defined as the minimum dosage that gives high fertilisation and hatching success, are similar for hormone-induced Australian percichthyids (Table 8). Our study supports the findings of early researchers who showed that mature *M. novemaculeata* will not ovulate without the use of hormones (van der Wal, unpublished data 1983; Battaglene et al. 1989a).

The optimal dosage of 500 I.U.kg^{-1} hCG for *M. novemaculeata* is the same as that of *M. ambigua* (Rowland 1983). However, unlike *M. ambigua,* not all mature *M. novemaculeata* ovulated and spawned when induced with optimal doses of hCG (Table 7; Rowland 1983). Induced *M. novemaculeata* which ovulated but did not spawn generally produced fewer inferior quality eggs (Figs 3,4 and 5). This may be because *M. novemaculeata* are cyclical spawners, and only part of the complement of yolky oocytes can be obtained at a single stripping (Harris 1986). However, multiple stripping yields poorly fertilised eggs and it is more likely that initial stripping is taking place prior to the completion of ovulation.

The 38 h period used in this study before intervention and stripping is 4 h after the mean spawning time of 34 h. The stripping time represents a compromise between allowing

broodfish time to complete ovulation and spawning, and egg quality deterioration. A delay of even a few hours in stripping ovulated eggs of most warm-water fishes can greatly reduce the success of fertilisation (Rees and Harrell 1990; Shelton 1989; Scott, Zeldis & Pankhurst 1993). For example, the eggs of *Morone saxatilis* an American anadromous spring-spawning species, must be stripped within 1 h of ovulation, and Murray cod *Maccullochella peelii*, an Australian percichthyid, should be stripped within 2 and 6 h of ovulation (Stevens 1966; Rees and Harrell 1990; Rowland 1988). Further research is required to determine post-ovulatory egg viability in *M. novemaculeata*.

Van der Wal (in Rowland 1983) reported that the latency period after injection of 500 $I.U.kg^{-1}$ hCG in *M. novemaculeata* was significantly longer than the latency period after injection of 1000 or 1500 $I.U.kg^{-1}$ hCG. This result lead the Port Stephens Research Centre hatchery to adopt 1000 $I.U.kg^{-1}$ as the optimum dosage for routine spawning. However, these early experiments were confounded by fluctuating water temperatures and no increase in latency period with 500 $I.U.kg^{-1}$ was found in the current study. In fact, all captive females injected with 500 $I.U.kg^{-1}$ spawned within 38 h and had fertilisation rates >80% in the low dosage rate trial (Table 5).

The period when female *M. novemaculeata* can be hormone-induced to spawn is between three and four months, with males running ripe before females are mature. The length of the breeding season in the wild is shorter around three months and the availability of wild broodfish depends on geographic location, latitude and river discharge (Harris 1986). Pond-held fish can be used to extend the breeding season, particularly when early winter flooding stimulates spawning in wild broodstock.

Atresia and ovarian involution is common in wild Australian bass towards the end of the breeding season if flood stimuli are lacking (Harris 1986), as shown by the increased proportion of stage IV oocytes in wild fish compared with captive fish (Table 3). Fish starting ovarian involution produced fewer fertilised eggs and induced-ovulation was less predictable. Similar problems with induced spawning at the end of the breeding season have been described for related species *Macquaria ambigua, Maccullochella macquariensis* and *Morone saxatilis* (Rowland 1983; Ingram and Rimmer 1992; Curry Woods & Sullivan 1993).

In the wild there is partial segregation of the sexes, with male Australian bass remaining in estuarine habitats (Harris 1986). It can therefore be difficult to simultaneously obtain equal numbers of both sexes. The ability to induce females in the absence of males (Table 7) will facilitate controlled breeding experiments, hybridisation and assist hatcheries to maintain genetic diversity by allowing cross fertilisation of individual males with more than one female. It will also assist hatcheries to breed fish collected from different locations, an important consideration given the concern over translocation of Australian native fish between river

drainages (Harris & Battaglene 1990).

Our study shows that hatcheries can use either wild or captive fish and the choice depends on availability, transport distance, season, and river conditions. The small number of fish that died during the study clearly demonstrates that collection by gill netting is efficient but as with the collection of *Morone saxatilis* (Yeager, Van Tassel & Wooley 1990), care must be taken to avoid physically damaging the fish. If possible broodfish should be collected in freshwater and spawned in saltwater to reduce stress and assist in the control of disease and parasites (Yeager et al. 1990; Selosse and Rowland 1990; Rowland and Ingram 1991).

CONCLUSION

Commercial production of Australian bass in eastern Australia relies solely on hormone-induced breeding. The techniques described in this paper will increase the large-scale production of Australian bass by providing optimal hormone doses, and determining spawning and stripping times, for both wild and captive fish. Improved husbandry practises will increase the quality and quantity of larvae produced while minimising production costs and the quantity of wild broodstock collected.

ACKNOWLEDGMENTS

We thank the staff of the Port Stephens Research Centre for their assistance. In particular, we thank Mr P Beevers, Mr M Lockhart and Mr B Talbot for fish collection, hormone induction, stripping and data collation; Mr P Beevers for drawing the figures; and Ms J Pickle for typing the manuscript. Dr G Allan, Mr P Beevers, Mr B Ingram, Dr B Nowak, Assoc Prof N Pankhurst, Dr S Rowland and Mr B Talbot provided helpful comments on an earlier draft.

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Table 1. The number of captive females injected during June, July, August and September 1984 to 1990. Fish from June, July and August 1984 to 1986 were used in dosage rate trials Experiment 1. Fish from 1990 were used in low dosage rate trial Experiment 2.

	Year							
Month	84	85	86	87	88	89	90	Total
June				8	8	5	16	37
July	12	14	16	2	5			49
August	24	15	14		6	1		60
September	7		2			3		12
Total	43	37	40	7	11	4	16	158

Table 2. The number of wild-caught females injected each month for the period 1987 to 1991.

	Year						
Month	87	88	89	90	91	92	Total
April			4				4
May	7	12	9	4			32
June	7	4	7	5	8	12	43
July			6	2	10	12	30
August	11		3				14
Total	25	20	25	11	18	24	123

Table 3. Percentage of oocyte stages in ovarian samples taken from captive and wild Australian bass prior to injection of hCG. Captive samples taken from 1984 to 1986, wild samples from 1987 to 1990.

	April		May		June		July		August		September	
	C	W	C	W	C	W	C	W	C	W	C	W
Source (n)	(0)	(4)	(0)	(32)	(37)	(30)	(45)	(25)	(60)	(14)	(12)	(0)
[1]Oocyte Stage												
I Primary oocytes	-	100	-	16.3	3.1	4.3	37.6	1.2	14.4	0	0	-
II Yolk vesicle yolk globule	-	0	-	83.7	95.5	85.0	51.7	81.6	47.4	33.6	45.8	-
III Yolk mass	-	0	-	0	1.4	10.7	10.7	17.2	33.3	47.9	51.7	-
IV Artretic	-	0	-	0	0	0	0	0	4.9	18.5	2.5	-

[1] For further details of stages see text
C Captive
W Wild

Table 4. Effect of different doses of hCG on spawning, stripping, ovulation, fertilisation and hatching success of Australian bass eggs. The captive fish were injected in June, July or August 1984 to 1986.

	Number of females			Number of females producing fertilised eggs %		Mean fertilisation[1] rate ± SE %		Number of females producing hatched larvae %		Mean hatch[1] rate ± SE %	
Dose	Spawned	Stripped	Failed to Ovulate	Spawned	Stripped	Spawned	Stripped	Spawned	Stripped	Spawned	Stripped
500[2]	14	7	6	12(85.7)	6(85.7)	95.6±3.5	84.1±8.9	10(71.4)	6(85.7)	66.6±8.9	81.2±5.3
1000	31	8	12	21(67.8)	6(75.0)	92.9±3.9	72.5±11.7	16(51.6)	5(62.5)	78.5±5.8	60.7±15.7
2000	16	4	3	13(81.3)	3(75.0)	96.1±2.2	83.7±12.0	11(68.8)	3(75.0)	87.2±2.7	38.2±19.4
Total	61	19	21	46	15			37	14		
%	60.3	18.8	20.8	75.4	78.9			60.7	73.7		

[1] No significant difference existed in mean fertilisation and hatch among doses of 500 to 2000 u/kg ($P>0.05$) for spawned or stripped fish.
[2] Not including two females which died.

Table 5. The effect of low dosages of hCG on the ovulation, spawning, fertilisation and hatch rate of captive Australian bass in June 1990.

hCG IU.kg^{-1}	Arbitrary oocyte stage %			Mean oocyte diameter (mm ± SE)	Oocyte maturation	Ovulation	Spawning	Fertilisation rate (%)	Hatch rate (%)	Mean hatch rate ± SE (%)
	I	II	III							
0		90	10	0.9±0.03	-	-	-			
0		100		0.9±0.01	-	-	-			
0		100		0.9±0.03	-	-	-			0
0	5	95		0.9±0.02	-	-	-			
100		100		0.9±0.02	-	-	-			
100	5	90		0.9±0.02	-	-	-			
100		100		0.9±0.01	+	+	+	1	1	0.25±0.3
100		100		0.8±0.01	+	+	-			
200	10	85	5	0.9±0.03	+	+	+	97	94	
200	10	90		0.8±0.03	+	+	+	0	0	
200	5	90	5	0.9±0.02	+	+	+	3	0	
200		100		0.9±0.02	+	+	+	0	0	23.5±23.5
500	10	90		0.8±0.03	+	+	+	99	92	
500	10	90		0.8±0.02	+	+	+	99	98	
500		100		0.9±0.02	+	+	+	98	97	
500		100		0.9±0.02	+	+	+	81	52	84.8±11.0

Table 6. Monthly comparisons of mean fertilisation and hatch rates of spawned and stripped Australian bass eggs from wild fish injected with 1000 I.U.kg^{-1} hCG.

Month (Number)	Result	Number	Number females producing fertilised eggs	Mean[1] fertilisation rate ± SE%	Number trials with hatched larvae	Mean hatch[2] rate ± SE%
April (3)	Spawned	1	1	96	0	-
	Stripped	0				
	No ovulation	2				
May (25)[3]	Spawned	9	7	97.3±1.2	7	88.6±6.5
	Stripped	5	5	86.9±6.3	5	81.9±8.8
	No ovulation	11				
June (14)	Spawned	7	6	94.8±2.1	6	74.7±12.3
	Stripped	5	5	69.2±12.2	5	51.5±16.2
	No ovulation	2				
July (6)	Spawned	1	1	97	1	96
	Stripped	4	4	58.0±13.1	1	95
	No ovulation	1				
August (3)	Spawned	0				
	Stripped	0				
	No ovulation	3				
Total	Spawned	18 (35 %)	15	96.2±1.0	14	77.7±8.3
	Stripped	14 (28 %)	14	71.7±6.3	11	69.2±9.5
	No ovulation	19 (37 %)				

[1] Does not include fish which failed to produce fertilised eggs.
[2] Does not include fish which failed to produce larvae.
[3] Not including one fish which died.

Table 7. Mean fertilisation and hatch rates for wild-caught Australian bass, *Macquaria novemaculeata* injected with 500 I.U.k^{g-1} hCG in June and July 1990 - 1992 a) in the presence of males b) without males

		Number of females	Number females producing fertilised eggs	Mean[1] fertilisation rate ± SE%	Number trials which hatched	Mean hatch[2] rate ± SE%
a)	Spawned	21	19	89.5±4.6	16	88.2±5.3
	Stripped	10	10	83.2±3.9	8	71.8±5.2
	No ovulation	3				
b)	Spawned	2	0			
	Stripped	7	7	70.0±23.4	7	61.9±8.5
	No ovulation	0				

[1] Does not include fish which failed to produced fertilised eggs.
[2] Does not include fish which failed to produce larvae or those terminated due to very low (<10%) fertilisation rates.

Table 8. Comparison of optimum dosages and latency periods for Australian percichthyids injected with human chorionic gonadotropin (hCG)

Species	Female optimum dosage ($I.U.kg^{-1}$)	Female range induce ovulation & spawning ($I.U.kg^{-1}$)	Latency period (h)	Male dosage ($I.U.kg^{-1}$)	Temperature (°C)
Macquaria ambigua[1]	500	500-2000	26-38	200	25°C
Macquaria australasica[2]	750	750-1000	38.8-42.8	200	18-20
Macquaria novemaculeata[3]	500	500-4000	34.2 mean	200	18-20
Macquaria colonorum[4]	Unknown	500-1000	34-40	200	18-20
Maccullochella peelii[5]	1000	1000 or 2000	48.5-49.5	500	20-22
Maccullochella macquariensis[6]	Unknown	1000-3000	57.4 mean	500-1500	20

1 Captive fish (Rowland 1983)
2 Induction of wild fish with hCG (Gooley and McDonald 1988)
3 Current study captive fish
4 Wild fish (Battaglene unpublished data 1988)
5 Captive fish Rowland (1988)
6 Captive fish Ingram & Rimmer (1992)

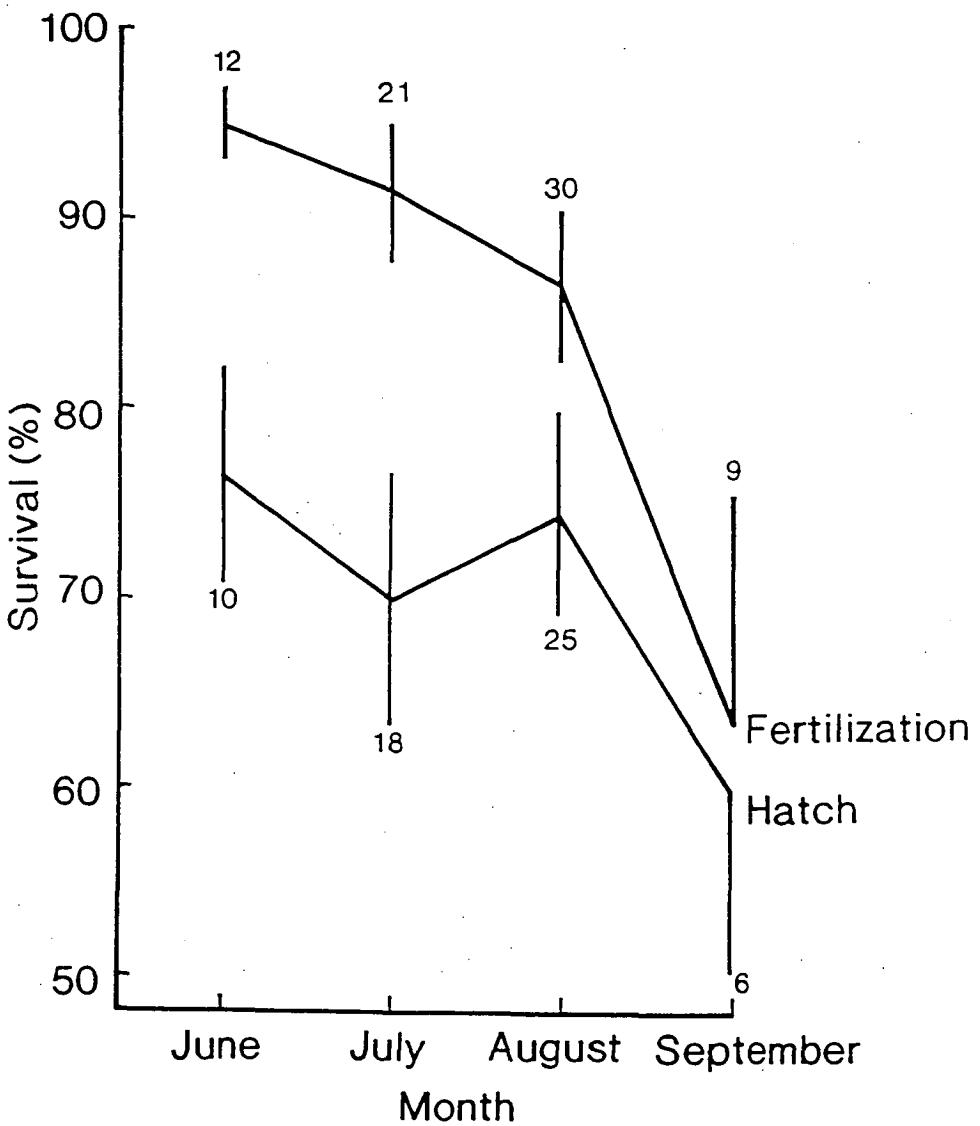

Figure 1 The mean monthly (1984-1986) fertilisation and hatch rates for captive *Macquaria novemaculeata* injected with dosages of 500-4000 I.U.kg^{-1} hCG. Points represent means; bars are standard errors.

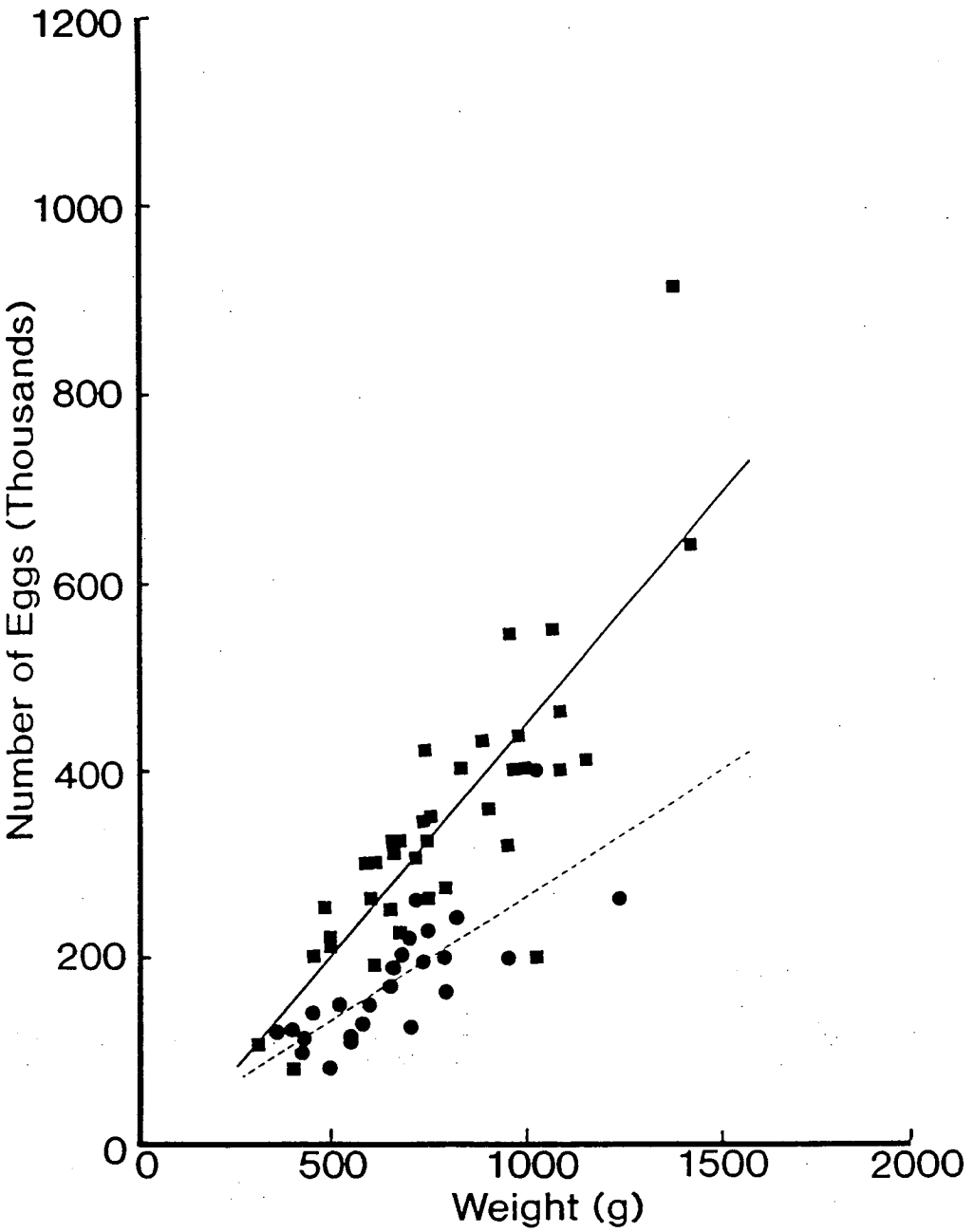

Figure 2 Relationship between the weight (WT) of wild female *Macquaria novemaculeata* and the number of eggs (NE) from spawned fish (■), NE = -49724 + 497WT(g), (n=36, R^2=0.69,P<0.0001) and stripped fish (●), NE = -1395 + 266WT(g), (n=24, R^2=0.61,P<0.0001). Data for females injected with 500 or 1000 I.U.k^{g-1} hCG in May, June and July, excluding those which produced unfertilised eggs.

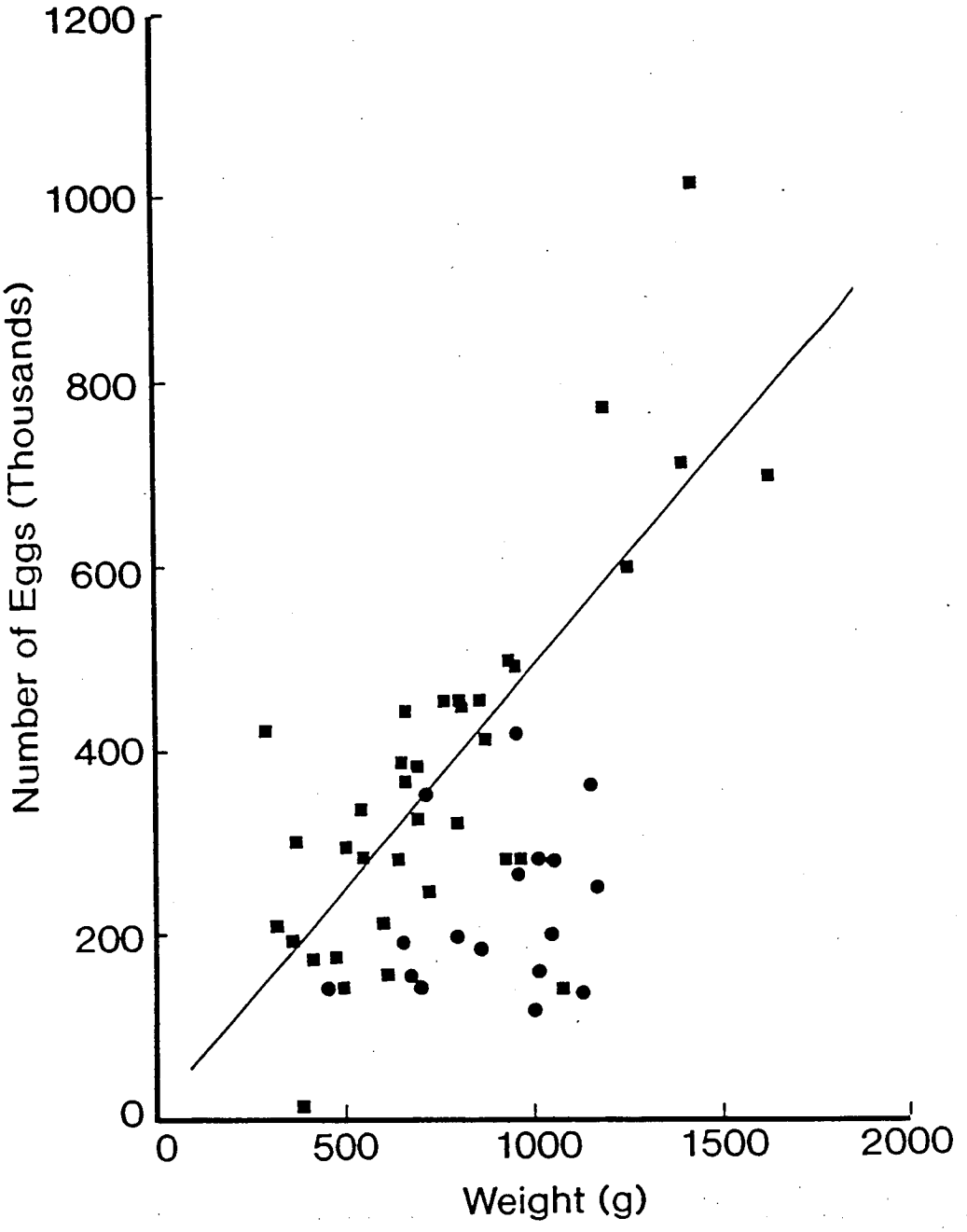

Figure 3 Relationship between the weight (WT) of captive female *Macquaria novemaculeata* and the number of eggs (NE) from spawned fish (■), NE = 11087 + 480WT(g), (n=36, R^2=0.58,P<0.0001) and stripped fish (●). Regression line for spawned females only. Data for females injected with 500 or 1000 I.U.kg^{-1} hCG in June, July and August excluding those which produced unfertilised eggs.

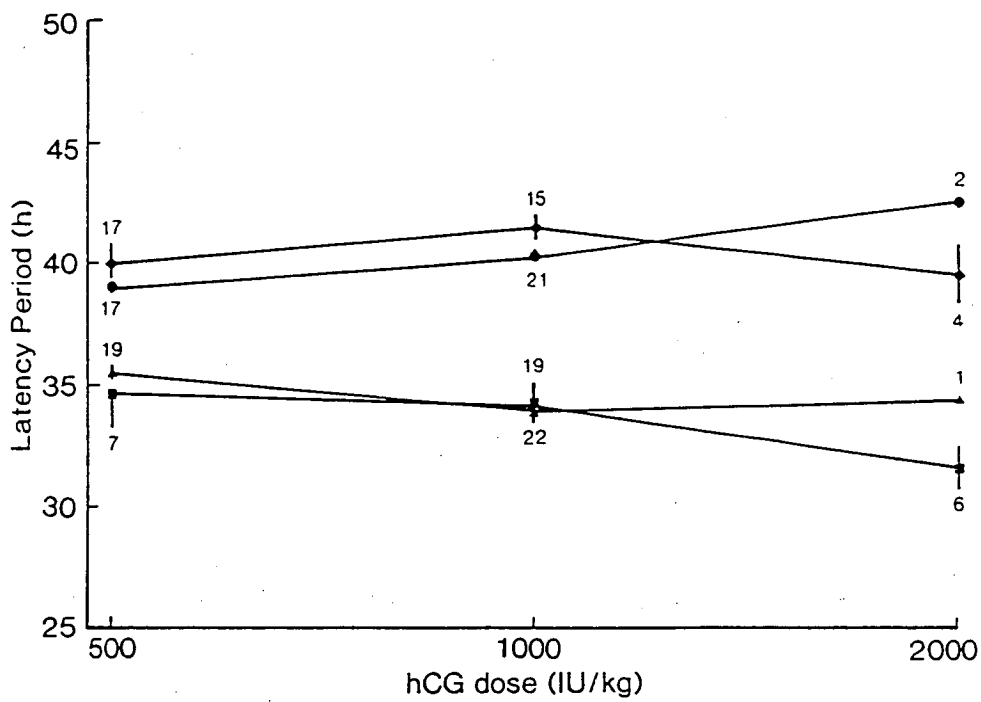

Figure 4 Mean latency period for captive and wild Australian bass *Macquaria novemaculeata* injected with 500,1000 or 2000 I.U.kg^{-1} hCG. Data is for all years except 1984 and 1987 and only includes fish which produced fertilised eggs. Points represent means; bars standard errors; n=number of fish; ◆ stripped captive fish; ■ captive fish which spawned; ● stripped wild fish; ▲ wild fish which spawned.

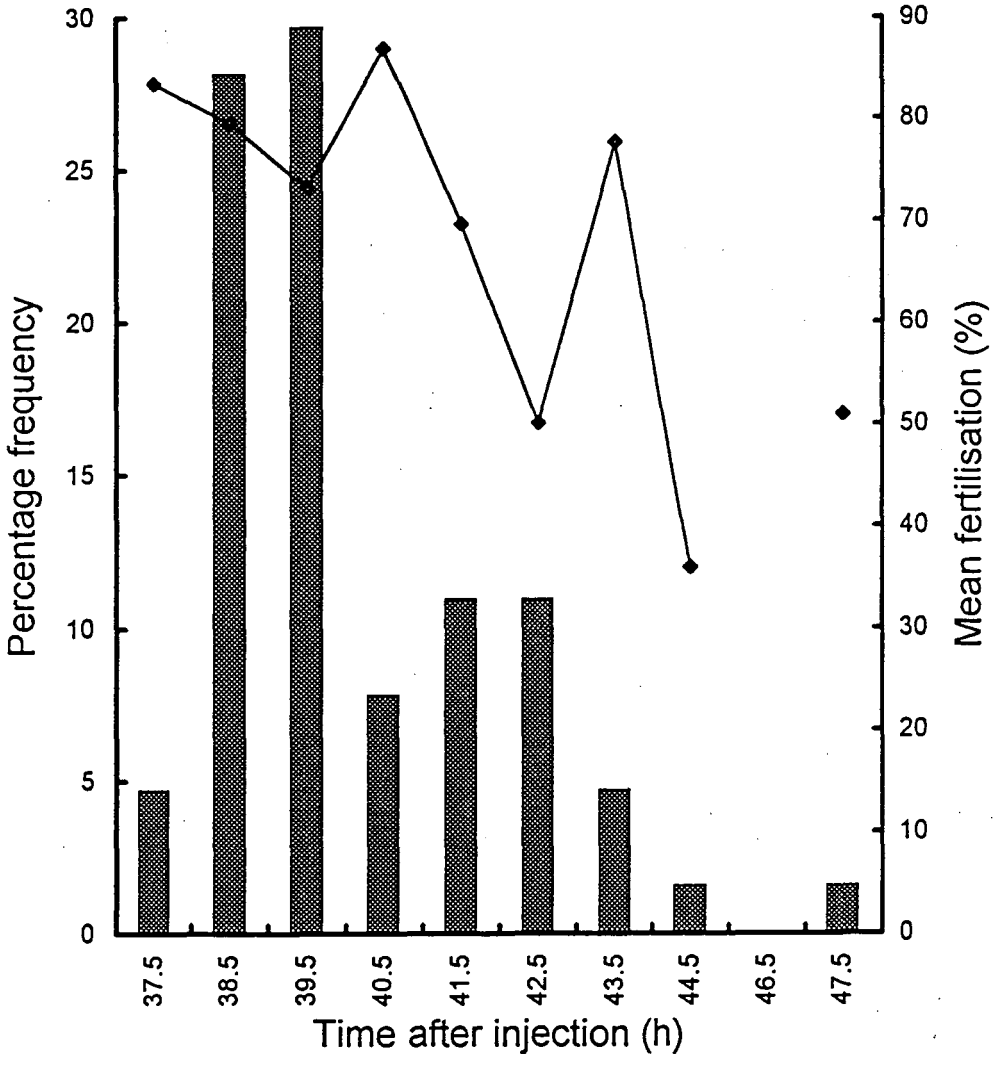

Figure 5 Percentage frequency histogram of the time period between injection and successful stripping for fish that ovulated but did not spawn, showing the mean percentage fertilisation for each time interval. Data are for wild and captive fish injected with 500 or 1000 I.U.kg^{-1} hCG, n=64.

CHAPTER 3

Initial swim bladder inflation in intensively reared Australian bass larvae, *Macquaria novemaculeata* (Steindachner) (Perciformes:Percichthyidae)

Stephen C Battaglene and R Bill Talbot

t is the published article: Battaglene, S. C., Talbot, R. B., 199 . nitial swim bladder inflation in intensively reared Australian bass larvae, acquaria novemaculeata (Steindachner) (Perciformes: Percichthyidae), Aquaculture, 86(4), 431-442

Published in Aquaculture

CHAPTER 4

Effects of Salinity and aeration on survival of and initial swim bladder inflation in larval Australian bass

Stephen C Battaglene and R Bill Talbot

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t is the published article: Battaglene, S. C., Talbot, R. B., 1993. ffects of salinity and aeration on survival of and initial swim bladder inflation in larval Australian bass, The progressive-fish culturist, (1), 3 -39

Published in the Progressive Fish-Culturist

CHAPTER 5

Supplementary feeding with brine shrimp, *Artemia*, in the extensive brackish water culture of Australian bass *Macquaria novemaculeata* (Steindachner)

Stephen C Battaglene, R Bill Talbot and Geoffrey L Allan

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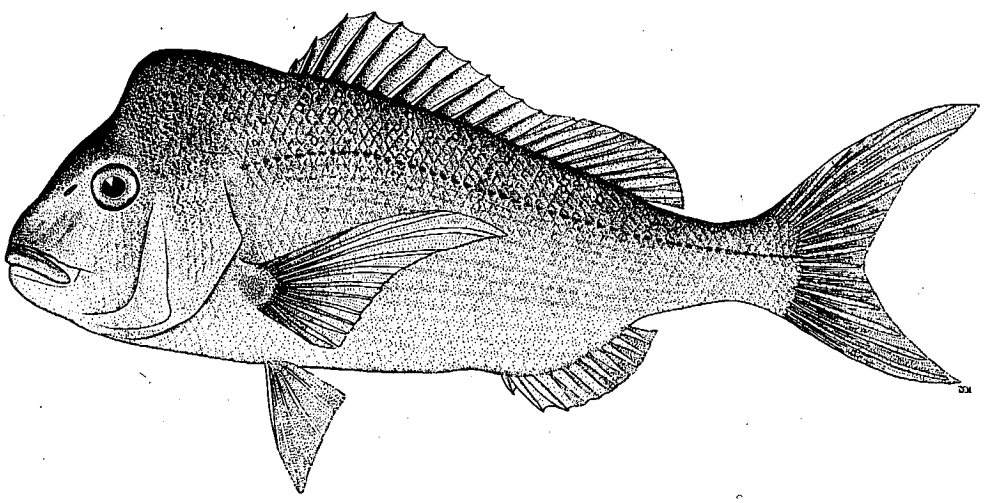
t is the published article: Battaglene, S. C., Talbot, R. B., Allan, . ., 1992. Supplementary feeding with brine shrimp, Artemia, in the e tensive brac ish water culture of Australian bass, acquaria novemaculeata (Steindachner). n: . . Allan and . all (ditors), Proc. Aquaculture utrition or shop, Salamander Bay, 1 -17 April 1991. S isheries. Brac ish ater ish Culture Research Station, Salamander Bay, Australia, pp. 197-198.

Published in The Proceedings of the Aquaculture Nutrition Workshop

PLATE 2 Snapper, *Pagrus auratus*
(Drawn by Jack Hannon)



CHAPTER 6

Induced spawning and larval rearing of snapper, *Pagrus auratus* (Pisces: Sparidae) from Australian waters

Stephen C Battaglene and R Bill Talbot

t is the published article: Battaglene, S. C., Talbot, B. T., 1992, nduced spawning and larval rearing of snapper Pagrus auratus (Pisces: Sparidae), from Australian waters, ew ealand ournal of marine and freshwater research 26(2), 179-183

Published in New Zealand Journal of Marine and Freshwater Research

CHAPTER 7

Development of hatchery techniques for snapper, *Pagrus auratus* in Australian

Stephen C Battaglene and Geoffrey L Allan

t is the published article: Battaglene, S. C., Allan, . ., 1994. evelopment of hatchery techniques for snapper, Pagrus auratus, in Australia. n: . . Chou et al. (ditors), Proceedings of the Third Asian isheries orum, anila 1992, pp. 1 8-111.

Accepted for Publication in

Proceedings of the Third Asian Fisheries Forum

CHAPTER 8

Induced ovulation of captive and wild snapper, *Pagrus auratus* (Sparidae) using hCG, LHRHa and Ovaprim

Stephen C Battaglene

Submitted to the New Zealand Journal of Marine and Freshwater Research

ABSTRACT

Australian snapper *Pagrus auratus* were captured by trapping and long-lining, and returned to the laboratory. Eighteen trapped female fish were injected with either 500 or 1000 $U.kg^{-1}$ hCG. Three out of six females injected with 500 $U.kg^{-1}$ hCG and 8 of 12 females injected with 1000 $U.kg^{-1}$ ovulated. Individual females were assessed every 8 h and successfully stripped up to five times. Females produced viable eggs up to 100 h after injection. The highest fertilisation occurred at 24±4 h and 40±4 h post injection. Three long-lined females injected with 1000 $U.kg^{-1}$ hCG all ovulated and one 3.2 kg fish produced 558,656 eggs in 7 batches with fertilisation ranging from 8 to 98 %. Two experiments were also conducted with captive snapper. HCG induced ovulation in 4 out of 6 captive females with oocytes > 560 µm, although latency periods were longer (69-91h). In a separate experiment, the effects of hCG injection, LHRHa pellet implantation or injection with 'Ovaprim', on maturation were compared in captive females. Implantation with LHRHa pellets induced ovulation in all five females treated (mean oocyte diameters of 464±42 µm), from 163 to 211 h after implantation. Whereas injection with hCG failed to induce ovulation in five females with mean oocyte diameters of 498±29 µm, and only one of five fish treated with Ovaprim ovulated. The cyclical production of eggs, approximately every 24 h, from long lined fish injected with hCG and captive females treated with LHRHa is consistent with reports for New Zealand and Japanese *P. auratus* indicating that spawning occurs on a daily basis. The main problem with obtaining eggs using hormone induced fish is pinpointing the time of ovulation because the period of post-ovulatory egg viability is short.

Keywords snapper; *Pagrus auratus;* ovulation ; LHRHa; hCG; Ovaprim; aquaculture

INTRODUCTION

One of the current limitations to the expansion of snapper *Pagrus auratus* culture in Australasia is the supply of good quality eggs. Hatcheries presently obtain fertilised eggs from snapper collected from the wild which are naturally ovulating, or hormone-induced to ovulation, or from spontaneous spawnings of captive fish held in large public aquariums (Smith 1986; Pankhurst et al. 1989; Battaglene & Talbot 1992; Anon 1993). In New Zealand and Australia, several attempts have been made to get wild caught snapper to spawn in captivity in tanks or pools under ambient conditions, but while snapper feed and grow in captivity they also rapidly develop gonadal atresia soon after capture and will not subsequently ovulate or spawn (Bell et al. 1991; Carragher & Pankhurst 1991; Pankhurst & Carragher 1992; Battaglene unpubl. data). Furthermore, supplies of snapper eggs from aquariums can be erratic and the eggs obtained can be mixed with eggs from other species (Battaglene unpubl. observation).

The best opportunity for obtaining a reliable supply of eggs therefore appears to be from fish injected with hormones. Spawning has been induced in wild-caught *P. auratus* following injection with human chorionic gonadotropin (hCG), both in New Zealand (Pankhurst et al.1991; Pankhurst & Carragher 1992) and Australia (Battaglene & Talbot 1992; Battaglene & Allan in press). However, not all mature *P. auratus* injected with hCG ovulate, and those that do ovulate often produce poor quality eggs, with low survival and fertilisation rates (Pankhurst et al 1991; Battaglene & Talbot 1992; Pankhurst & Carragher 1992; Battaglene & Allan in press). In Japan, farmed *P. auratus* can be induced to spawn with hCG one month prior to the breeding season (Lopez 1986), but hormone treated wild fish produce poor quality eggs (Foscarini 1988). Some success has also been achieved in the out-of-season spawning of domesticated *P. auratus* using luteinizing hormone-releasing hormone analogue (LHRH-a) pellets, however, the eggs produced were also of poor quality (Matsuyama et al. 1993).

One of the problems with obtaining eggs from hormonally induced females is pinpointing the time of ovulation because of the short-term viability of ovulated eggs in the oviduct prior to stripping (Scott et al. 1993: Battaglene & Allan in press). Other factors also contribute to the poor quality of hormone-induced eggs including, the stress of capture and handling (Carragher & Pankhurst 1991; Pankhurst & Sharples 1992). Studies in New Zealand and Japan have shown *P. auratus* are repeat serial spawners, spawning in spring on a daily basis (Foscarini 1988; Scott & Pankhurst 1992; Matsuyama et al 1993; Scott et al. 1993). There is little information available on the reproductive biology of snapper in Australia but it is reasonable to assume that a similar reproductive profile exists for snapper in Australian waters.

Ultimately, the solution to the supply of snapper eggs will probably come by holding domesticated stock in photoperiod and temperature controlled tanks. In Japan, pen-reared and tank-held red sea bream, a reproductively isolated northern population of *Pagrus auratus* (Paulin 1990), have been domesticated for over nine generations and spawn naturally in captivity (Fukusho et al. 1986, Fukusho 1990; Foscarini 1988). However, there will remain a need for eggs from wild fish for many years and possibly indefinitely if juveniles are to be released in stock enhancement programs.

In the present study, the percentage fertilisation and number of eggs produced from wild-trapped and long-lined *P. auratus* injected with hCG and stripped at varying time intervals was determined. In addition, two experiments were performed on wild snapper caught as juveniles and held in captivity for five years. In the first experiment, the effects of single or multiple doses of hCG on ovulation in captive fish were compared. In the second, the effects of three different hormones, hCG, LHRHa and 'Ovaprim' (a proprietary mixture of LHRHa and the dopamine antagonist domperidone) on maturation were assessed.

MATERIALS & METHODS

Capture and maintenance of fish

Wild snapper were obtained from baited traps at 20 to 60 m depth off Broughton Island, New South Wales (32° 38'S, 152° 15'E) in August to November 1993. Fish handling and transportation were as described by Battaglene & Talbot (1992). A small number of snapper were also captured using baited long-lines in the same area where the fish were trapped.

Laboratory acclimated wild fish were held in a pen in the marine research pool at the Fisheries Research Institute at Cronulla (34° 47'S, 151° 8'E). The fish were collected as young-of-the-year in 1989 and used in growth trials (Bell et al. 1991). The fish were maintained on a diet of chopped pilchards (*Sardinops neopilchardus*) and pellets (Bell et al. 1991) and had been disturbed by seine netting in April of 1991 and 1992 for a measurement of length and weight of some fish. The water temperature in the pool varied seasonally from 14 to 24°C.

Fish handling and sampling

Wild fish were anaesthetised with ethyl p-aminobenzoate (50 mg l^{-1}). Males were examined for the presence of expressible milt and a sample of oocytes was obtained from females via a glass 1 ml pipette inserted into the ovary through the oviduct. The diameter of the ten largest oocytes obtained was measured for each female. Females were then injected or implanted with hormones as described below. Males were injected with 500 or 1000 U /kg body weight hCG to increase milt volume (Pankhurst 1994). Following hormone treatment, male and female fish caught in the wild were placed together in a 5000 l circular tank with continuously flowing saltwater (30 l min^{-1}), at a salinity of 35‰ and temperature of 20±1°C. The fish were

kept in the dark to minimise external disturbance and removed at regular intervals, anaesthetised, and stripped. For further details on handling see Battaglene & Talbot (1992).

Laboratory acclimated snapper were seine netted from the pool, tagged with Floy anchor tags and then treated in the same manner as wild fish. Following hormone treatment, fish were transported within 12 h to the NSW Fisheries Research Centre at Port Stephens, in 750 l containers with oxygen 40 ml min^{-1}. The fish were transferred to 10 000 l tanks with dip nets and examined approximately every 8 h for ovulation. Ovulating fish were anaesthetised and stripped. The various hormone treatments were stocked into separate tanks. A maximum of five females and three males were stocked into any one tank. Egg traps were used on the outlets of the tanks to determine if the fish spawned between ovulation checks.

Stripped eggs were collected in 200 ml of saltwater and fertilised by milt obtained from stripped males. Eggs were incubated in 70-litre glass aquaria with aerated (100 l air min^{-1}), continuously flowing sea water at 21.5±1°C. Fertilisation rates were determined by microscopically examining about 100 eggs per incubator just prior to hatch. Water temperature was measured daily, whereas salinity, dissolved oxygen, and pH were measured every 3 days.

Hormone treatment

The preparation and administration of hormones followed the methods of Rowland (1983) and Lee et al. (1986). Wild-trapped snapper were treated with a single intraperitoneal injection of human chorionic gonadotropin (hCG) at 500 or 1000 U /kg body weight. Females were initially treated with 1000 $U.kg^{-1}$ hCG, the dosage previously used to induce ovulation in snapper (Pankhurst et al. 1991; Battaglene & Allan in press). However, as the number of ovulating females being caught increased, a reduction in hCG to 500 $U.kg^{-1}$ hCG was tested on some females. All wild-caught females with oocytes > 450 µm were treated with hormones.

Two experiments were conducted with laboratory acclimated snapper. In the first experiment, the effects of single and multiple doses of hCG on maturation were examined. Six mature females with oocytes >500µm were injected with 1000 $U.kg^{-1}$ hCG and three of these were injected with a further dose of 500 $U.kg^{-1}$ hCG 48 h later. The experiment was conducted in September 1993.

The need for eggs to satisfy hatchery production quotas, combined with the erratic and relatively small number of mature wild females available for experiments, prevented the use of saline injected control females. However previous experience suggested that hormone treatment is required unless females are ovulating at capture.

In the second experiment, the effects of three different hormones, hCG, lutenizing hormone-

releasing hormone pellets (Des Gly^{10} [D-Ala^{6}]-LHRH-ethylamide) and Ovaprim {(D-Arg^{6}, Pro^{9}-NEt) sGnRH with domperidone}, on maturation of laboratory acclimated females was examined. Five females were treated with 1000 U.kg^{-1} hCG, five with LHRH-a pellets and five with 0.5ml/kg ovaprim(10mg sGnRH and 5mg domperidone/kg). The various treatments were allocated randomly and treatment groups were stocked into tanks each with three spermiating males. The experiment was conducted in October 1993.

Slow release LHRH-a cholesterol pellets were prepared using a modification of the method described by Lee et al. (1986). Two milligrams of LHRH-a (Sigma Chemical Company, St Louis, USA, Product no. L-4513) were dissolved in 400 µl 50 % ethanol and mixed into 45 mg of cholesterol (Sigma Chemical Company, Product No. C-8867). The mixture was dried for one hour at 35 °C and then thoroughly blended with approximately 40 mg of melted copha. The mixture was then weighed into ten equal portions and packed into a PVC plastic mould with 12x2 mm diameter holes. The resulting 6x2 mm pellets contained 200µg LHRH-a and were implanted through a 2mm needle into the dorsal musculature of the fish.

Spermiating male fish collected from the wild were injected with 250 to 1000 U.kg^{-1} hCG. Captive males produced more milt than males collected from the wild and were not injected with hormones.

Statistics

Homogeneity of variances was evaluated using Cochran's test (Winer 1971). A nested one-way ANOVA was used in experiment 2 to separate differences in the initial mean diameter of oocytes within and among treatments. Where significant differences were found means were compared by SNK tests (Underwood 1981). Paired t-tests were used to test for changes in the mean oocyte diameter of females treated with Ovaprim and hCG (Winer 1971).

RESULTS

Hormone induction of wild fish caught in traps

Forty three female and 42 male wild snapper were caught in traps. Twenty three of the females had non-vitellogenic ova, 16 had vitellogenic ova and 4 ovulated eggs which were successfully stripped. Mature females were caught from the beginning of August to the middle of December (Table 1). The smallest mature female weighed 700g and its length was 308 mm. The mean size of the largest oocytes varied from 480 to 1200 µm in fish injected with hCG (Table 1).

Three out of the six females injected with 500 U.kg^{-1} hCG and 8 out of the 12 females injected with 1000 U.kg^{-1} ovulated. Individual females were successfully stripped up to 5 times and produced viable eggs up to 100 h following injection. The total number of eggs stripped and the quality of eggs varied greatly between fish and stripping times (Table 1 and Fig. 1). The

highest mean percentage fertilisation occurred at 24±4 h and 40±4 h (Fig. 2). However, the smallest quantity of eggs was obtained at 24±4 h and high fertilisation rarely coincided with the production of large numbers of eggs.

Snapper, particularly those over 2 kg were often damaged by the traps and some had severe scale loss. Overripe oocytes were observed in 4 of the 18 trapped snapper at capture and appeared hydrated, opaque and irregular in shape. Similarly, many batches of stripped eggs were opaque, irregular in shape and non-floating. The poor condition of repeatedly stripped females usually terminated trials. Gonads from sacrificed fish usually contained further batches of vitellogenic and hydrated eggs.

Ten immature male snapper were caught and the smallest male with viable sperm weighed 600 g and its length was 302 mm. The volume of milt available from running ripe males declined towards the end of the breeding season. Testes removed from badly damaged fish and kept in the fridge at 5°C retained viable sperm for at least 24 h.

Hormone induction of wild fish caught using long lines

Only three mature females were caught using long lines but all ovulated following injection of 1000 $U.kg^{-1}$ hCG and produced viable eggs. The female caught in October 1992 weighed 3.2 kg, had mean oocytes of 550±20 µm, produced 558 656 eggs over 90 h and was stripped 7 times over 5 days (Fig. 3). There was evidence of a 24 h ovulation cycle and in contrast to most of the trap-caught snapper, there was a positive relationship between the number of eggs stripped and percentage fertilisation (Fig. 3). The other females were caught in November and December and had mean oocyte diameters of 1170±30 µm and 1000±40 µm, respectively. Both were stripped four times producing 176,165 eggs (fertilisation 0-96 %) and 56,372 eggs (fertilisation 0-31 %), respectively.

Hormone induction of laboratory acclimated snapper

In experiment 1, all females had mean oocyte diameters greater than 560 µm prior to injection with hCG, but none could be stripped during the first 48 h following injection. Two of the three fish given a second dose of 500 $U.kg^{-1}$ hCG ovulated and one of the single injection fish produced viable eggs. Latency periods ranged from 69 h for the single injection female to 92 h for females given a second resolving dose (Table 2).

In experiment 2, there was no difference in the mean oocyte diameters for fish among hormone treatments groups prior to treatment but individual fish had mean oocyte diameters ranging from 408-616 µm (Table 3). All the fish injected with LHRH-a pellets ovulated but did not spawn. The two females with the largest initial oocytes produced a single large stripping of overripe eggs after 163 h. Two other females produced large batches of fertilised eggs from 170 to 226 h after injection (Table 3). Maximum egg production and fertilisation occurred

around 180 h after implantation in the latter two females(Fig. 4). Of the Ovaprim treated fish, only the female with the largest mean diameter oocytes (616±48 µm) at the start of the trial ovulated after 66 h and was successfully stripped (Table 3). The remaining females treated with Ovaprim showed no significant change ($t=1.11$, $df=3$, $P>0.05$) in mean oocyte diameters after 96 h. Similarly, none of the fish injected with hCG ovulated within 96 h, and there was no significant change ($t=1.58$, $df=4$, $P>0.05$) in the mean oocyte diameters following treatment.

DISCUSSION

HCG has commonly been used to induce ovulation in a wide range of fish (Donaldson & Hunter 1983). For example, the sparids *Sparus aurata, Archosargus probatocephalus, Dentex dentex, and Puntazzo puntazzo* have all been induced to spawn with doses of hCG ranging from 100 to 1800 $U.kg^{-1}$ (Zohar & Gordin 1979; Faranda et al. 1985; Tucker & Barbera 1987; Glamuzina et al. 1989).

Studies with *S. aurata* indicate they are sensitive to low hCG and LHRHa doses and depression of egg production can occur with moderate increases in hormone dose (Zohar & Gordin 1979; Colombo et al. 1989). In *P. puntazzo* doses of over 1000 $U.kg^{-1}$ hCG caused either the release of overripe eggs or retention of hydrated oocytes (Faranda et al. 1985). *Pagrus auratus* has been induced to ovulate using a relatively narrow range of hCG doses from 500 to 1000 $U.kg^{-1}$ (Lopez 1986; Pankhurst et al.1991; Battaglene & Talbot 1992; Pankhurst & Carragher 1992).

The presence of hydrated oocytes in sparids treated with hCG indicates a batch of oocytes is in the final stages of maturation, however, it is not a clear sign of development towards spawning (Faranda et al. 1985). Indeed, the presence of large (>1000 µm) hydrated oocytes in trapped snapper often indicated the retention of overripe oocytes which were slightly opaque and irregular in shape. The viability of ovulated snapper oocytes decreases with residence times over 8 h and possibly earlier (Scott et al. 1993). Decreases in viability in eggs of other species are associated with morphological, biochemical and physiological changes (McEvoy 1985; Kjorsvik et al. 1990). The timing of stripping in relation to ovulation is therefore critical and emphasises the advantage of induced spawning over manual stripping.

Variation in egg quality is probably also related to the effects of stress. The effects of stress on male and female snapper following capture, handling and confinement has been the focus of research in New Zealand (Carragher & Pankhurst 1991; Pankhurst & Sharples 1992). Short-term stress affects plasma levels of sex steroids and cortisol, and long-term stress inhibits ovulation in female snapper in New Zealand (Carragher & Pankhurst 1991, 1993). It therefore appears likely that the deteriorating condition of broodstock due to the stress of being in the trap, for up to 48 h, is an important factor affecting ovulation and egg quality. The potential

role of stress factors in the differential response to hCG treatment (see below) is less clear. Pankhurst & Carragher (1992) showed that there was no difference in plasma cortisol levels between fish that either did or did not respond to hCG treatment.

Another possible cause of poor quality eggs in laboratory acclimated fish and to a lesser extent wild fish is poor nutrition. For example, daily changes in the diet of domesticated red sea bream greatly affects the quality of eggs during natural spawning (Watanabe et al.1984; Foscarini 1988). Of particular importance is the level of essential fatty acids, fat soluble vitamin E and carotenoids (Watanabe et al.1984; Watanabe 1985). Improved captive broodstock nutrition at the Fisheries Research Institute may increase the percentage of ovulating females, fertilisation and larval survival.

The injection of hCG triggered repeat spawning in 43% of *S. auratus* for up to 100 days following injection with hCG (Zohar & Gordin 1979). Pankhurst & Carragher (1992) found that long-lined snapper treated with 1000 $U.kg^{-1}$ hCG ovulated only once before gonadal atresia started. This was not generally the case with trapped or long-lined snapper in the current study which were successfully stripped up to seven times (Table 1).The different responses exhibited by snapper in New Zealand and Australia probably reflect differences in husbandry and handling, particularly the separation of the sexes in the New Zealand study (Pankhurst & Carragher 1992). However, the possibility that significant differences exist between the two populations should not be ignored.

Only 11 of the 18 mature snapper ovulated following hCG treatment (Table 1). Similar response ratios to hCG are described by Pankhurst & Carragher (1992). However, 13 of the 18 trapped females used in the present study had started ovulation prior to hormone treatment. It is therefore difficult to determine what effect if any the hCG had in these females. As mentioned in the materials and methods section, production constraints did not allow saline injected control females. However, some support for the contention that hCG is responsible for increasing the number of ovulated eggs and repeat ovulation is available from other studies. During a separate study, in November 1994, twelve trapped females were held for periods of 24 to 168 h without hormone treatment in the presence of spermiating males. None of the females spawned but eight had from 5 to 70% hydrated oocytes (Table 4, J. Cleary, unpublished data, 1994).

The cyclical production of eggs from long-lined fish (Fig. 3) and captive females induced to spawn following treatment with LHRHa (Fig. 4) supports studies in Japan and New Zealand indicating that snapper are daily spawners (Foscarini 1988; Scott & Pankhurst 1992; Matsuyama et al 1993; Scott et al. 1993). The production of multiple batches of eggs from trapped snapper provides further support for this spawning pattern (Table 1).

The exact timing and location of spawning in wild snapper is unknown in Australia. The successful stripping and fertilisation of eggs from trapped females prior to hormone treatment around 1600 h suggests that spawning takes place around dusk or early evening indicating a reproductive pattern displayed by *P. auratus* in Japan and New Zealand and a number of other closely related sparids (Zohar & Gordin 1979; Matsuyama et al. 1988; Kagawa et al. 1991; Scott et al. 1993).

Although there have been few detailed studies, single injections of LHRHa do not appear to be superior to hCG at inducing repeating ovulation in snapper. Pankhurst & Pankhurst (1989) found that only 44 to 66 % of snapper injected with LHRHa spawned. Battaglene & Allan (in press) found that females injected with Ovaprim had longer latency periods and produced more batches of eggs than fish injected with hCG. However, there was no overall increase in the viability or number of eggs produced from Ovaprim induced fish. It should be noted that Ovaprim contains the dopamine antagonist domperidone and while it has been successfully used in combination with LHRHa to induce ovulation in cyprinids (Peter et al. 1988), the usefulness of dopamine antagonists in marine teleosts is not clear. LHRHa has been demonstrated to be superior at inducing ovulation in *S. auratus* than hCG but the response was not enhanced by co-treatment with dopamine antagonists (Zohar et al. 1987; Colombo et al. 1989).

The results of the present study show LHRHa pellets are effective at inducing repeat ovulation in snapper with mean oocyte diameters of 464±42 µm (n=5) but the percentage of fertilised eggs was generally low (0 to 75 %) (Table 3). Matsuyama et al. (1993) found that single implantation of a copolymer pellets containing 100 µg LHRHa induced spawning in completely immature red sea bream 18 days after implantation. However, the percentage fertilisation of eggs was also low (12-46 %). While the reasons for the low fertilisation are unclear in both studies, overripening appears most likely with snapper, and insufficient maturation of the testes in red sea bream (Matsuyama et al. 1993). Further research is needed using LHRHa pellets in captive broodstock.

In summary, hormone-induced ovulation in trapped snapper is difficult to predict and incorrect stripping and or poor egg quality causes low fertilisation, poor hatch and ultimately low larval survival. Further research is required before all of the important criteria needed for assessing the effectiveness of induced ovulation are met (Peter et al. 1988). It is particularly difficult to predict when ovulation will occur in fish which have started final oocyte maturation. The use of snapper from traps set for short periods of time, changes in the timing of injection and the use of long-lined fish also need further investigation.

ACKNOWLEDGMENTS

I thank members of the NSW Fisheries Research Centre Port Stephens for assistance in the collection of broodstock. In particular, I thank P. Beevers, J. Cleary and B. Talbot for assistance with fish capture, injection and stripping of fish and production of LHRHa pellets. Ian Walker kindly provided the trapped fish and Jenny Cleary the data on control snapper. I also thank B. Nowak, W. O'Connor, and N. Pankhurst for constructive comments on the manuscript. This study was partially funded by the Fishing Research and Development Council of Australia.

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TABLE 1

Details of wild-caught trapped snapper *Pagrus auratus* injected with human chorionic gonadotropin (hCG) a) 500 U.kg b) 1 000 U.kg^{-1}

Date	Weight (g)	Oocyte[1] (mean±SD) (µm)	No. of eggs[2]	Number of batches	Fertilisation (%)	Hatch (%)
a						
10-11-93	2 200	1200±130[3]	78 400	4	0-86	0-99
10-11-93	700	RR[3]	23 951	4	0-91	0-99
12-11-93	1 200	950±30	77 046	5	0-18	0
24-11-93	5 400	1120±40	-	-	-	-
24-11-93	2 000	930±30	-	-	-	-
24-11-93	2 700	920±30	-	-	-	-
b						
9-8-93	700	980±40[3]	16 875	3	45-87	0-98
9-8-93	650	510±30	-	-	-	-
9-8-93	750	480±20	-	-	-	-
23-8-93	1 200	550±30	104 616	5	9-69	5-99
23-8-93	2 400	640±60	-	-	-	-
7-9-93	1 500	970±30	-	-	-	-
7-9-93	2 200	RR[3]	16 032	1	94	UN
7-9-93	2 050	580±50	6 072	3	18-76	UN
1-11-93	750	980±60	1 000	2	0-88	0
3-11-93	700	970±50	19 200	3	0-31	0
3-11-93	800	1090±50	84 800	2	0-6	0
24-11-93	2 300	1120±60	7 590	1	0	-

[1] Mean oocyte size at injection
[2] Total fecundity for all batches of eggs
[3] RR=Running ripe at capture
UN Unknown

TABLE 2

Captive snapper injected in September 1993 with a) 1 000 U.kg^{-1} hCG b) 1 000 U.kg^{-1} hCG and a further injection of 500 U.kg^{-1} hCG after 48 h

Weight (g)	Mean oocyte diameter (µm±SE)	Oocyte maturation	Ovulation	Batches of stripped eggs (<1 000)	Fertilisation rate (%)	First* latency period (h)	Number of eggs
a							
2 455	590±12	+	+	1	0	91	<100
1 684	604±13	+	+	4	1-22	69	50 600[+]
1 262	562±10	-	-	-	-	-	-
b							
1 610	601±22	+	+	1	83	44**	22 770
2 064	570±10	+	+	2	1-33	31**	78 430
1 800	642±14	-	-	-	-	-	-

\+ One batch UN
* Time between injection and first stripping
** 2nd injection of 500 U.kg^{-1} hCG at 48 h, latency period given are 2nd injection

TABLE 3

Ovulation, fertilisation and no. of eggs from captive snapper *Pagrus pagrus* injected with either 500 U.kg^{-1} hCG, LHRHa pellets or 0.5 ml Ovaprim.

Treatment	Weight	Mean oocyte diameter (µm)	Oocyte maturation	Ovulation	Batches of stripped eggs	Fertilisation rate (%)	First* latency period (h)	number of eggs
hCG	1 920	500±34	-	-	-	-	-	--
hCG	1 450	547±15	-	-	-	-	-	-
hCG	1 440	481±42	-	-	-	-	-	-
hCG	1 380	485±37	-	-	-	-	-	-
hCG	1 820	477±45	-	-	-	-	-	-
LHRHa	1 450	450±26	+	+	8	0-56	177	153 696
LHRHa	1 550	454±44	+	+	1	13	211	5 940
LHRHa	1 810	408±32	+	+	5	1-75	170	199 986
LHRHa	2 000	516±32	+	+	1	0	163	253 506
LHRHa	1 400	493±30	+	+	1	0	163	147 752
Ovaprim	2 000	447±32	-	-	-	-	-	-
Ovaprim	1 820	543±61	-	-	-	-	-	
Ovaprim	1 550	450±32	-	-	-	-	-	-
Ovaprim	1 300	466±22	-	-	-	-	-	-
Ovaprim	1 650	616±48	+	+	3	1-16	66	132 066

TABLE 4

Wild-caught snapper *Pagrus pagrus* retained in captivity without hormone treatment (Data provided by J. Cleary).

Hours in tank (h)	Fish weight (g)	Gonad weight (g)	Mean oocyte[1] diameter (µm)	Hydrated oocytes (%)
24	537	10	865±37	10
24	1750	46	862±60	5
24	286	11	874±36	20
24	715	9	502±36	0
24	678	4	473±33	0
24	1032	21	935±26	10
48	820	11	529±148	2
168	695	9.5	397±23	0
168	1985	54	883±21	30
168	822	8	815±46	50
168	491	5.8	849±53	70

[1] Mean ooctye diameter of the ten largest preserved oocytes

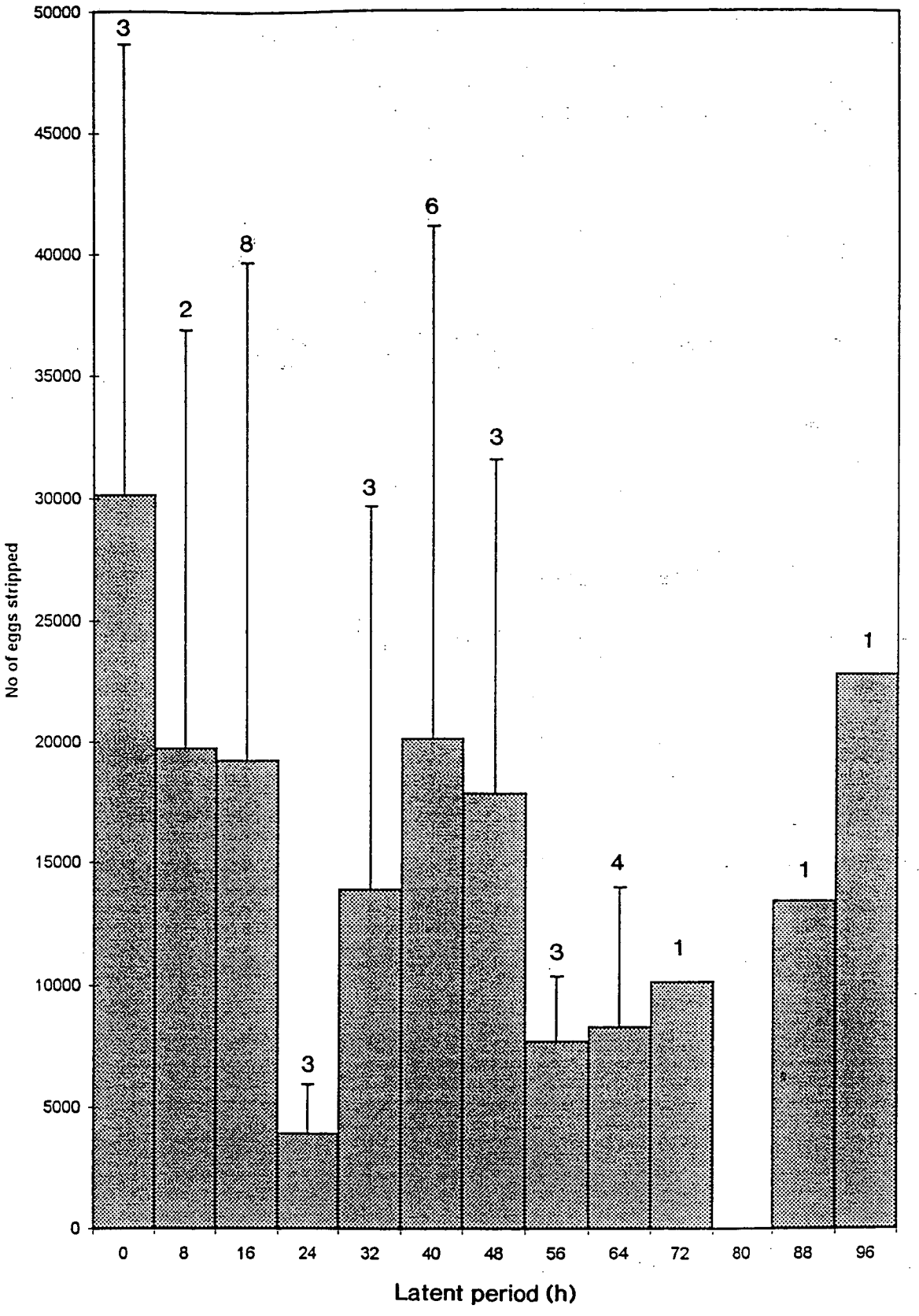


Figure 1

Frequency histogram of the mean number of eggs stripped from wild trapped snapper *Pagrus auratus* at capture and up to 96 h after injection with 1000 $U.kg^{-1}$ hCG. Bars indicate standard deviations and numbers above bars the number of females yielding eggs.

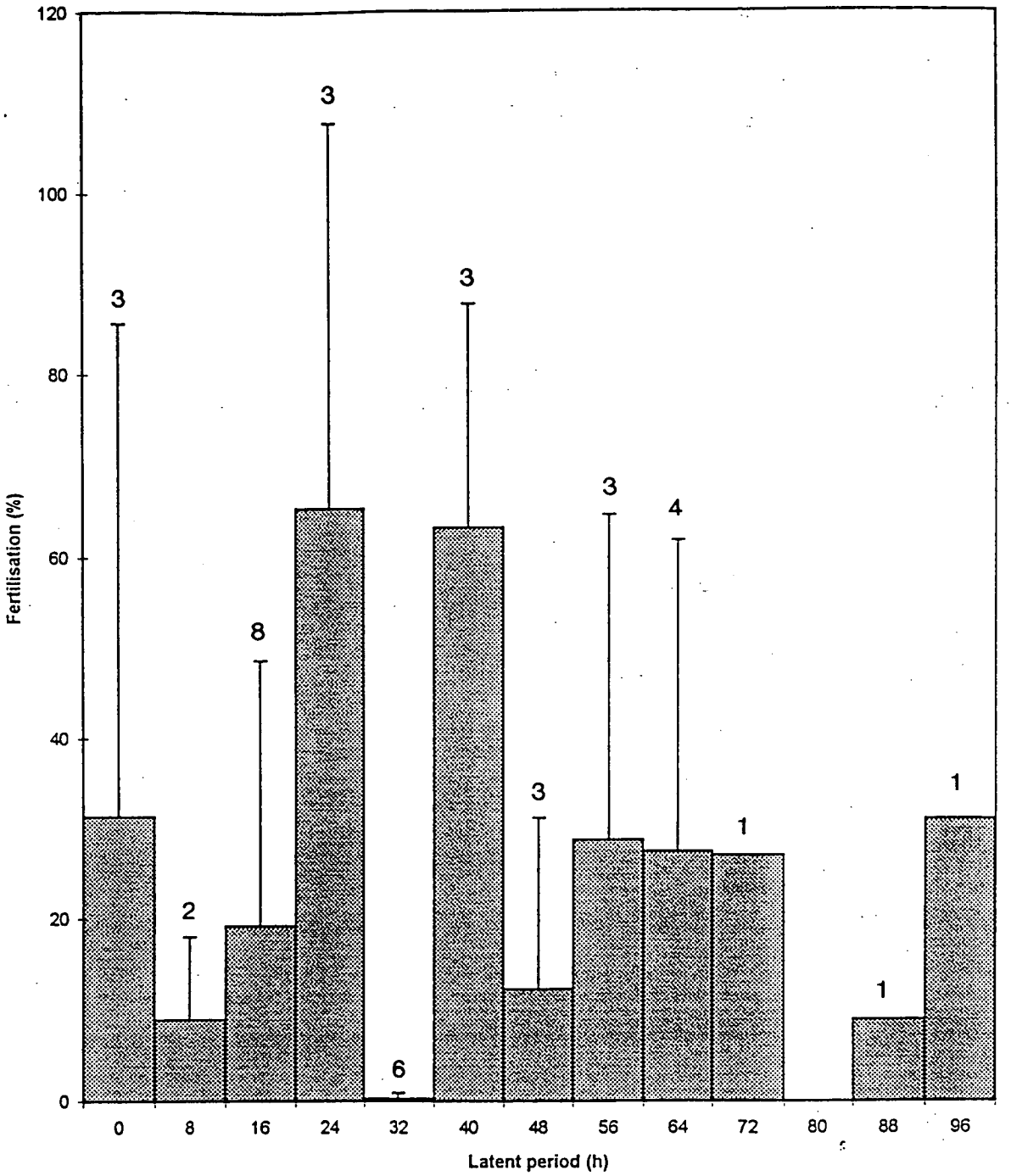


Figure 2

Frequency histogram of the mean percentage fertilisation of eggs stripped from wild trapped snapper *Pagrus auratus* at capture and up to 96 h after injection with 1000 U.kg^{-1} hCG. Bars indicate standard deviations and numbers above bars the number of females yielding eggs.

Fig 3

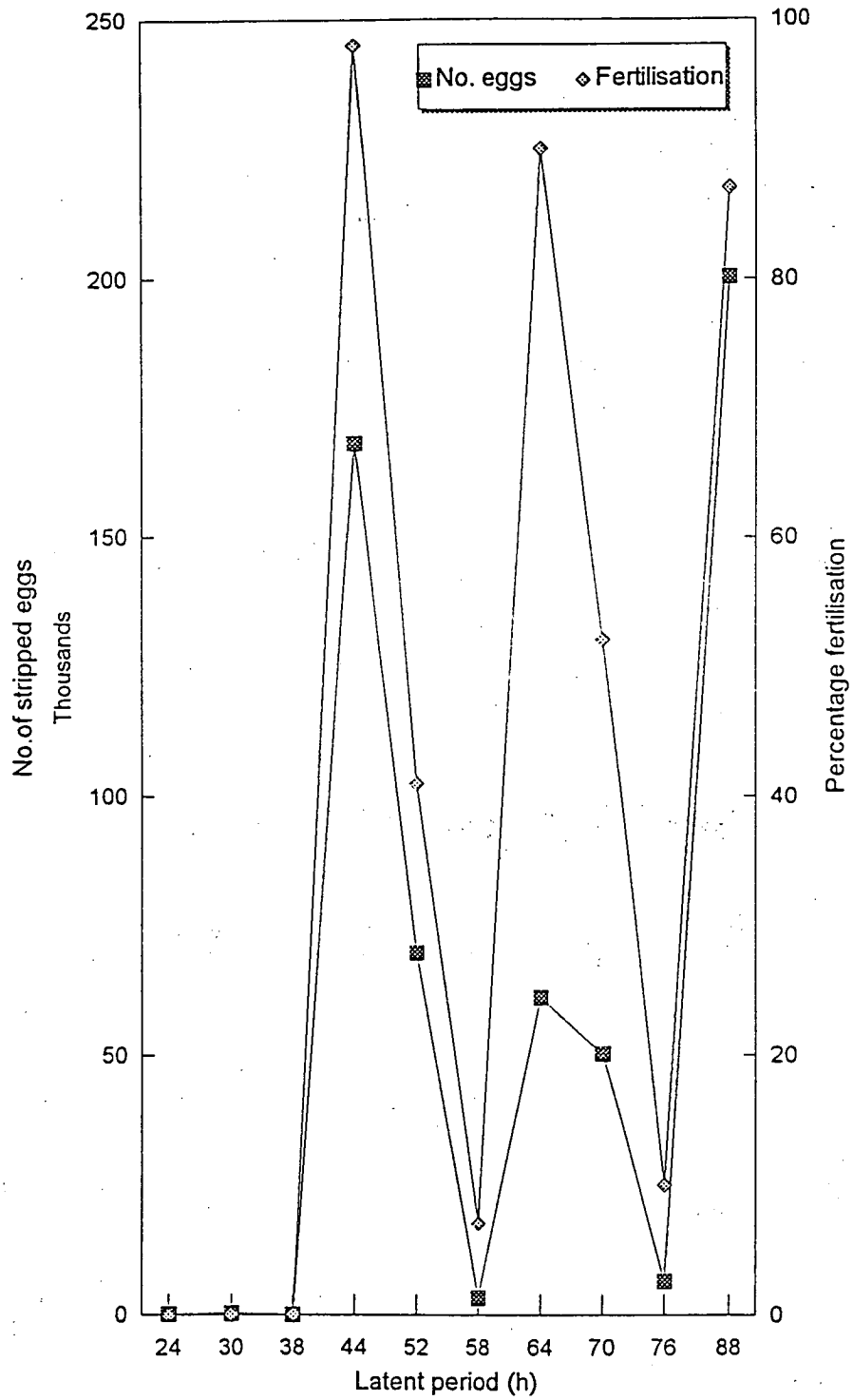


Figure 3

The number and percentage fertilisation of eggs stripped from a 3.2 kg long-lined snapper *Pagrus auratus* up to 88 h after injection with 1000 $U.kg^{-1}$ hCG.

Fig 4A

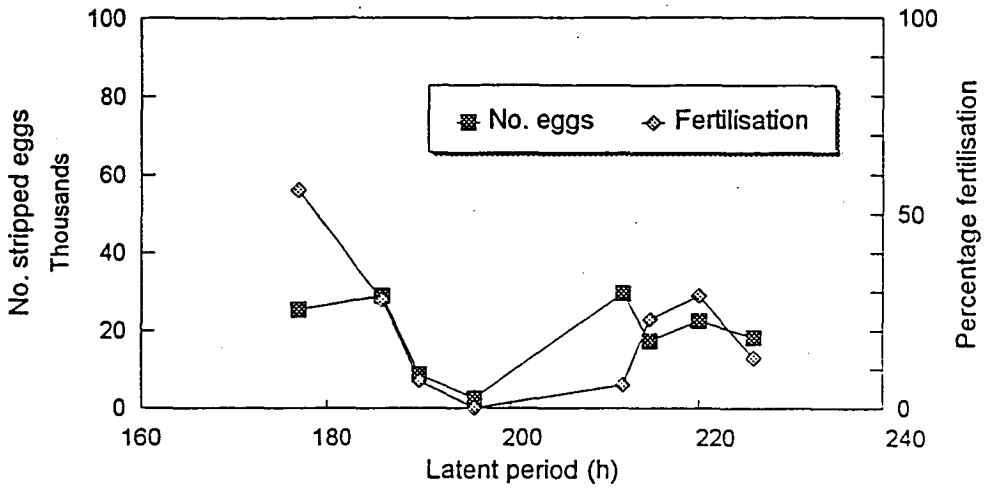


Fig 4B

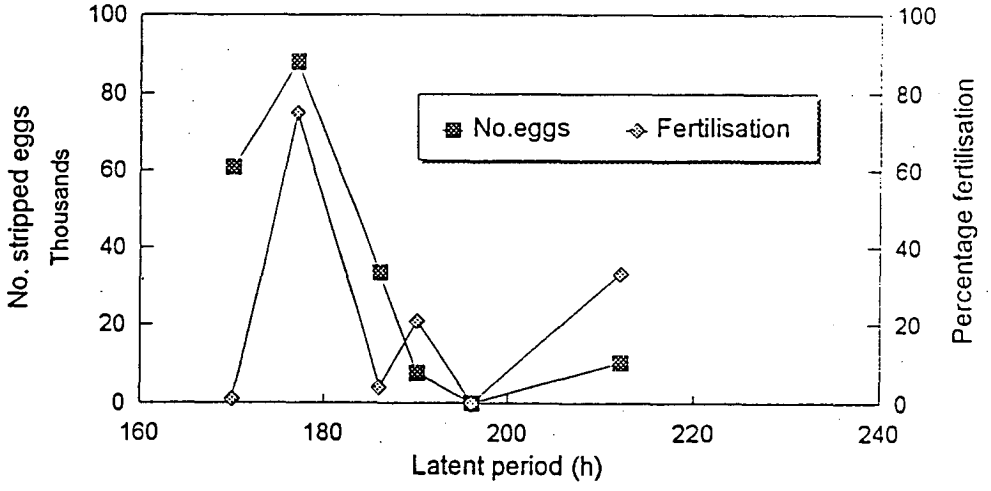
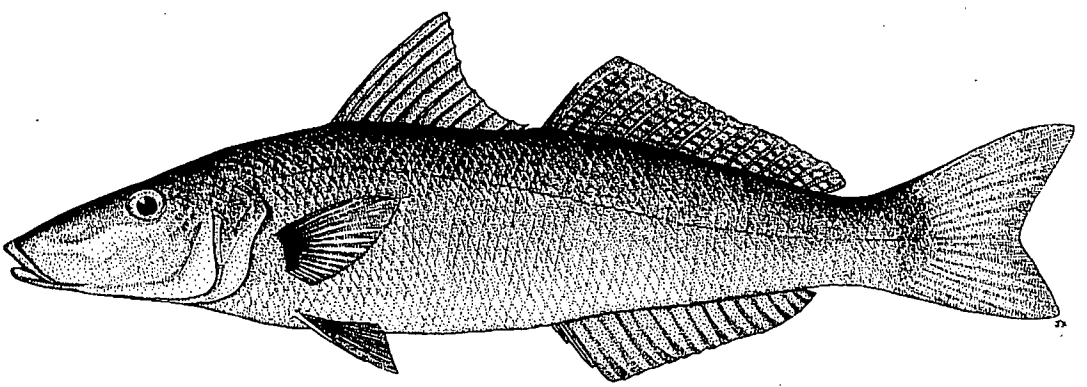


Figure 4

The number and percentage fertilisation of eggs stripped from A) 1.4 kg captive snapper *Pagrus auratus* B) 1.8 kg captive snapper, between 170 and 230 h after implantation with a LHRHa pellet.

PLATE 3 Sand whiting, *Sillago ciliata*
(Drawn by Jack Hannon)



CHAPTER 9

Hormone-induced ovulation of sand whiting *Sillago ciliata*

Stephen C Battaglene

Submitted to Asian Fisheries Science

ABSTRACT

Sand whiting, *Sillago ciliata* were captured by seine netting ocean beaches, and mature females were given single interperitoneal injections of 300 $U \cdot kg^{-1}$ human chorionic gonadotropin (hCG), or 0.5 $ml \cdot kg^{-1}$ Ovaprim (a synthetic GnRH analog with domperidone). One female and spermiating male were then placed in 70 l or 1000 l tanks. Both hCG and Ovaprim induced ovulation but not spawning in 38% and 75% of females, respectively (n=8). Females injected with distilled water (n=4) failed to ovulate. Fish were stripped and the eggs fertilised 32 to 48 h after injection and the mean percentage fertilisation was 85.6±15.4% (n=3, mean±SD) and 89.5±12.0% (n=6) in females injected with hCG or Ovaprim, respectively. Tank size had no apparent effect on the number of females that ovulated. A separate experiment was conducted to determine if naturally ovulating females could be successfully collected from the wild and stripped. The mean fertilisation in naturally ovulating females was 71.0±17.1% (n=4), lower than for Ovaprim injected mature fish collected at the same time (89.5±12.0%, n=2) but higher than in hCG injected fish (32.7±5.5%, n=3). The fertility of eggs from females injected with hCG was higher in the first experiment. Reasons for the different response to hCG between experiments were unclear, but the results suggest that Ovaprim is a more reliable hormone than hCG for inducing ovulation in sand whiting.

INTRODUCTION

The whitings or sand smelt of the genus *Sillago* (Family Sillaginidae) are commercially and recreationally important estuarine and near shore species of the Indo-west Pacific region (McKay 1985; Paxton et al. 1989). Members of the genus are frequently used in early life stage experiments because their biology and ecology in the wild is well understood and they are relatively small, daily spawners, commonly available in mature condition. For example, *Sillago japonica* and *Sillago sihama* have been used in Japan to study the effects of temperature and photoperiod on spawning, temperature and salinity on egg development, and the effects of steroids on germinal vesicle breakdown (Lee and Hirano 1985; Oozeki and Hirano 1985; Matsuyama et al. 1990; Furukawa et al. 1991). The supply of eggs usually comes from spawning fish either caught in the wild or held in captivity (Lee and Hirano 1985; Matsuyama et al. 1990). Alternatively, broodstock can be induced to ovulate and spawn with hormone injections (Lee et al. 1981; Oozeki and Hirano 1985).

The sand whiting, *Sillago ciliata* Cuvier is common in northern New South Wales and southern Queensland, where it is an important commercial and recreational species (McKay 1985). Sand whiting are highly fecund, multiple spawners with a prolonged spawning season extending from September to March (Morton 1985; Burchmore et al. 1988; Young 1991). Mature sand whiting have been successfully induced to ovulate using hormones and the larvae reared intensively (Young 1991; Battaglene et al. in press).

Reliable production of good quality fertilised eggs and larvae is required if sand whiting is to become a useful model experimental animal and if the suggested aquaculture potential of the species is to be assessed (Treadwell et al. 1992). Young (1991) successfully stripped captive sand whiting injected with 100 to 2700 $U \cdot kg^{-1}$ human chorionic gonadotropin (hCG) but no fish spawned, not all ovulated, and fertilisation of stripped eggs was highly variable.

The objectives of the current study were to determine if: i) wild-caught sand whiting ovulate ii) if non-ovulating fish can be induced to ovulate using hormones and iii) eggs produced from naturally ovulating and hormone-induced fish can be successfully fertilised.

MATERIALS AND METHODS

General

Two experiments were conducted with wild-caught sand whiting. Mature fish were seine netted from an ocean beach at Boat Harbour NSW, Australia (32° 47' 50" S 152° 6' 80" E) during summer (February 1992 and January 1993). Fish were transported to the Port Stephens Research Centre hatchery in a 750 l tank within one hour of capture and transferred to a 4,000 l tank with flow-through salt water. In the first experiment, fish were left for 24 h before being anaesthetised with p-aminobenzoate (50 $mg \cdot l^{-1}$) between 1400 and

1600 h. In the second experiment, fish were anaesthetised within 8 h of capture, between 2000 and 2200 h. Males were examined for expressible milt, and only spermiating males with very active sperm were used. A sample of oocytes was obtained from females by inserting a fine bore (1mm) silicone tube into the oviduct and aspirating tissue by mouth. The diameter of the ten largest oocytes was measured (±30μm) for each female. Fish were considered to have started final oocyte maturation if hydrated oocytes were detected.

Mature females which had not started final oocyte maturation and spermiating males were given a single interperitoneal injection with either 300 U•kg^{-1} human chorionic gonadotropin (hCG) (Pregnyl®, Organon [Australia] Pty Ltd) or 0.5 ml•kg^{-1} of Ovaprim®,[(D-Arg^{6}, Pro^{a} - NEt) a synthetic GnRH analog with a dopamine antagonist domperidone] (Syndel, Vancouver, Canada) dissolved in distilled water. Fish in control treatments were injected with 1 ml•kg^{-1} of distilled water. Fish were randomly allocated to treatments, with one female and male placed into either, 70 l glass aquaria with continuously flowing sea water, or 1,000 l static fibreglass tanks. Tanks and aquaria were contained in separate rooms and were kept in the dark, aerated, and maintained at a temperature of 23±1°C.

Experiment 1

The aim of the first experiment was to determine the effect of hCG and Ovaprim on ovulation in mature wild-caught females held in different holding systems. Eight replicate pairs of fish were treated with hCG, eight with Ovaprim and four with distilled water as controls. Four pairs of fish from each hormone treatment and the controls were placed in 1,000 l tanks and four pairs of hCG and Ovaprim treated fish in 70 l aquaria. Two different tank sizes were used to increase sample sizes and to determine if ovulation was influenced by the type of holding system used. Fish were anaesthetised at approximately 24, 32, 34, 36 and 48 h after injection and manually examined for signs of ovulation. If ovulation had occurred females were stripped.

Experiment 2

The aim of the second experiment was to compare ovulation, percentage fertilisation and hatch in hormone-induced females and ovulating wild-caught females. Four ovulating wild-caught females and four spermiating males were stripped and the eggs fertilised without hormone treatment. Four replicate pairs of fish were also treated with hCG, or Ovaprim and placed in 70 l aquaria. Hormone dosages were the same as in the first experiment and fish were anaesthetised after 34 h and stripped. Females were then examined every 30 minutes until 37 h after injection. Fish which failed to ovulate were then checked again 40 h after injection.

Stripped eggs were collected in 200 ml of sea water and fertilised by milt obtained from

stripped males. Eggs were incubated in 70 l glass aquaria with aeration (100 l air•min^{-1}) and continuously flowing sea water at 22±1°C. Fertilisation was determined by microscopically examining 100 or more eggs per incubator, two hours after stripping. The number of eggs per stripping was calculated volumetrically by counting five aliquots from each incubator. Eggs hatch in 24 h at 24±1°C (Battaglene et al. in press) so after 22 hours approximately 200 eggs were sampled from each incubator, the number of live embryos counted and the percentage hatch calculated.

Differences in ovulation in hormone treated females and the effect of holding tanks on ovulation were analysed using 2x2 contingency tables (Winer 1971). Separate one way analyses of variance were used to test for differences in female weight and mean oocyte diameter. Treatment means were compared using SNK tests. Homogeneity of variance was evaluated using Cochran's test.

RESULTS AND DISCUSSION

In the first experiment, 75% of females injected with Ovaprim, 38% of those treated with hCG and none of the fish in the control treatments ovulated (Table 1). Overall, there was no significant difference in the number of females which ovulated between fish injected with Ovaprim or hCG (χ^2 =2.29, df=1, P>0.05). Tank size had no apparent effect on the number of females which ovulated (χ^2 =0.25, df=1, P>0.05). Fish injected with Ovaprim were successfully stripped more often than those injected with hCG but subsequent strippings produced few, low fertility eggs (Table 1). Fish injected with Ovaprim generally ovulated before those injected with hCG and percentage fertilisation and hatch declined after 37 h in both treatments (Fig 1). The earlier stripping time and low fertility of eggs from multiple strippings suggests the fish injected with Ovaprim may have been stripped before they completed ovulation.

The percentage fertilisation of eggs stripped in experiment 1 was generally above 90% and higher than that achieved by Young (1991). In many species low fertilisation rates can result from over-ripening of oocytes, as they undergo morphological, biochemical and physiological changes, following ovulation (McEvoy 1985; Kjorsvik et al. 1990). The initial two low fertilisation values and all subsequent repeat strippings may be the result of over-ripening. Highest percentage fertilisation in experiment 1 occurred between 32 and 36 h, considerably earlier than the 48 h found by Young (1991). However, it should be noted that Young (1991) used captive fish with less developed oocytes, held at 21°C, compared with 23°C in the current study. Fertilised eggs floated and had a diameter of 700±20μm (mean±SD, n=10) with a single oil droplet, measuring 185±5 μm. Multiple oil droplets were present in some eggs from poorly fertilised batches. Further egg and larval development was as described by Tosh (1903) and Young (1991).

The failure of some fish to ovulate, and differences in the response to hormones did not appear to be related to variation in the size or maturity of the broodstock. The mean weight of females among treatments in experiment 1 was not significantly different ($P>0.05$) and only one female in the hCG (1, 000 L tank) treatment had significantly larger oocytes ($P<0.01$). The mean oocyte diameter among treatments was also not significantly different ($F=1.05$, $df=19$, $P>0.05$).

In experiment 2, all ovulating wild-caught females were successfully stripped without the use of hormones. Females injected with hCG produced larger numbers of eggs than naturally ovulating females or those injected with Ovaprim. However hCG injected females had lower percentage fertilisation and hatch (Table 2). The latent period for injected fish was narrower than in experiment 1, varying from 34 h to 36 h. One fish injected with hCG died and two treated with Ovaprim failed to ovulate within 40 h of injection.

The absence of control fish (non-ovulating and not injected) in experiment 2 was due to a shortage of mature females. It is possible that some of the females which could not be stripped at the start of experiment 2 may have ovulated without injection of hormones. However, control females in experiment 1 did not ovulate and we have not observed ovulation in sand whiting, during other hatchery trials, unless they can be stripped when captured (Battaglene, unpublished data 1993).

The mean fertilisation for hCG treated fish in experiment 2 was 32.7±5.5%, lower than the 85.6±15.4% in experiment 1. In contrast, the mean percentage fertilisation for Ovaprim treated fish in experiment 2 was 85.8±26.1% and similar to the 89.5±12.0% in experiment 1. Reasons for the different fertilisation rates using hCG but not Ovaprim between experiments were unclear but probably relate to the timing of stripping in relation to ovulation.

Seasonal differences and in the timing of hormone administration between the experiments may also be important in determining why fertilisation was low for hCG injected fish in experiment 2. Seasonal differences in maturity were possible, although not detected in the mean oocyte diameters, as experiment 1 was conducted in February 1992 and experiment 2 earlier in the spawning season in January 1993. Captive sand whiting collected towards the end of the breeding season in Queensland produced more fertilised eggs than those collected earlier in the season (Young 1991).

The 24 h delay in hormone injection in experiment 1 compared with less than 8 h in experiment 2 and the earlier timing of injection in relation to daylight in experiment 1 may also be important, particularly if sand whiting are daily spawners. The time of day when hormones are administered has been suggested to influence the success of ovulation in cyprinids (Peters et al. 1988), gilthead bream, *Sparus auratus* (Zohar 1988) and European sea bass *Dicentrarchus labrax* (Alvarino et al. 1992). It has been postulated that there is a daily change

in sensitivity either for the pituitary to LHRHa or for the ovary to gonadotrophin in some species (Allvarino et al. 1992).

The closely related Japanese whiting, *Sillago japonica* are known to have a circadian rhythm with respect to ovulation and production of 17α,20β-dihydroxy-4-pregnen-3-one, a possible maturation-inducing steroid, and spawning occurs between 1800 and 2400 h daily during the summer (Kashiwagi et al. 1987; Matsuyama et al. 1990; Furukawa et al. 1991). The exact location and timing of ovulation and spawning in sand whiting is unknown (Morton 1985; Burchmore et al. 1988).

Morton (1985) suggested sand whiting are multiple spawners on the basis of bimodal distributions in oocyte sizes, a protracted spawning season, and lack of synchronisation in mature fish. He also estimated fecundity to be highly variable and within the range of egg numbers produced by fish injected with hormones in the current study (Tables 1 and 2).

The results demonstrate that hCG and Ovaprim can induce ovulation in sand whiting. The protocol outlined using Ovaprim gave repeatable results and maturation time was short and predictable in ovulating females, resulting in good quality gametes. These features are some of the important criteria needed for assessing the effectiveness of induced ovulation (Peter et al. 1988). The response to hCG was not consistent between experiments, although hCG injected females generally produced more eggs than those injected with Ovaprim. Ovaprim therefore appears to be a more reliable hormone than hCG for inducing ovulation in sand whiting. However, the results should be interpreted with caution because not all sand whiting responded to hormone treatment and the number of females and the dosages tested were relatively small. Further research is required to determine optimum hormone dosages and if the dopamine antagonist domperidone is beneficial.

ACKNOWLEDGMENTS

I thank Paul Beevers, Bill Talbot, Joseph Taylor and Shannon McBride for technical assistance and Geoff Allan, Barbara Nowak, Wayne O'Connor, Ned Pankhurst and Stuart Rowland for useful comments on the manuscript.

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TABLE 1

Hormone induction data for wild-caught female sand whiting Sillago ciliata injected with distilled water, 300 U•kg^{-1} hCG or 0.5 ml•kg^{-1} Ovaprim and incubated in either 1000 l tanks or 70 l glass aquaria. (Experiment 1).

Treatment	Weight (g)	Mean Oocyte size mm±SD	Fertilisation (%)	Hatch (%)	No. [1] larvae	No. ovulated
Control	297	0.42±0.01	-	-	-	0/4
	266	0.44±0.01	-	-	-	
	200	0.42±0.02	-	-	-	
	230	0.46±0.02	-	-	-	
Ovaprim	300	0.43±0.02	-	-	-	3/4
(1 000 L tank)	252	0.44±0.01	33	10	464	
	215	0.42±0.03	97	96	39 987	
	274	0.44±0.01	98	96	55 193	
		2nd strip	42	10	800	
Ovaprim	248	0.44±0.02	97	94	17 005	3/4
(70 L tank)	229	0.44±0.02	92	83	22 399	
		2nd strip	0	0	0	
	175	0.42±0.01	-	-	-	
	260	0.42±0.03	97	95	24 522	
		2nd strip	59	36	3 802	
hCG	355	0.43±0.02	92	95	266 962	2/4
(1 000 L tank)	287	0.43±0.07	96	98	66 118	
	261	0.73±0.03	-	-	-	
	299	0.45±0.02	-	-	-	
hCG	248	0.45±0.02	68	65	104 486	1/4
(70 L tank)	229	0.43±0.02	-	-	-	
	205	0.43±0.02	-	-	-	
	218	0.44±0.01	-	-	-	

[1] Number of viable larvae = no eggs x percentage hatch

TABLE 2

Comparison of eggs produced by sand whiting Sillago ciliata, naturally ovulating and from fish injected with 300 U•kg^{-1} hCG or 0.5 ml•kg^{-1} Ovaprim. (Experiment 2).

Treatment	Weight	Mean oocyte size mm±SD	Fertilisation %	Hatch %	No. Eggs	No. Ovulating
Natural Ovulation	550	-	85	85	62 570	
	308	-	77	76	51 816	
	296	-	46	46	47 905	
	400	-	76	76	10 754	
MEAN±SD			71.0±17.1	70.8±17.0	43 261±2254	4/4
hCG	400	0.43±0.03	29	12	192 600	
	350	0.42±0.03	-	-	-	
	400	0.43±0.03	39	0	101 677	
	310	0.42±0.02	30	0	111 325[1]	
MEAN±SD			32.7±5.5	4.0±6.9	135 201±49	9433/4
Ovaprim	320	0.42±002	98	95	60 526	
	250	0.46±0.03	-	-	-	
	256	0.39±0.03	81	80	68 437	
	221	0.40±0.02	-	-	-	
MEAN±SD			89.5±12.0	87.5±10.6	64526±5530	2/4

[1] Including 38,000 unfertilised eggs collected from the aquaria

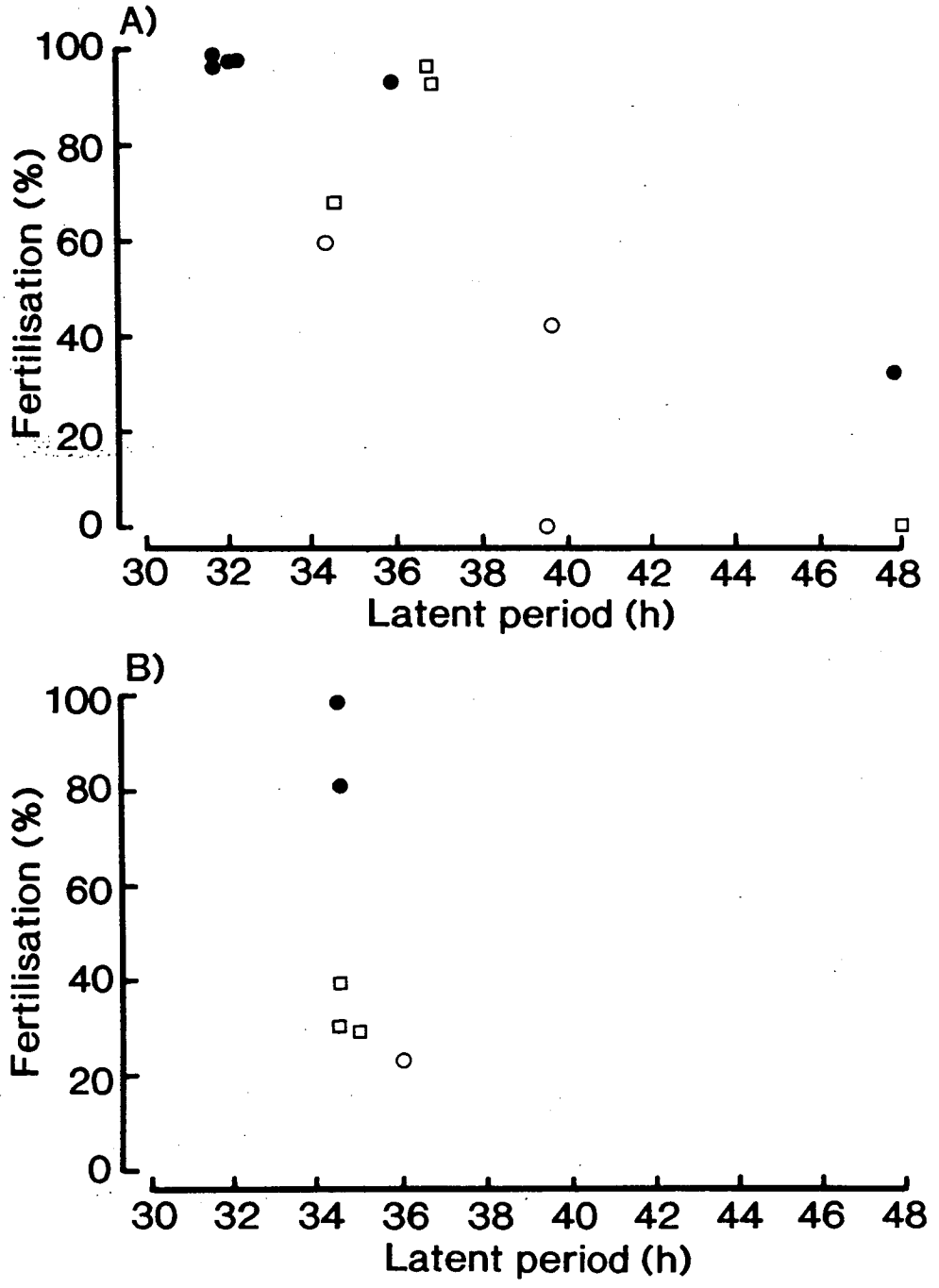


Figure 1. Latent period and mean fertilisation for sand whiting Sillago ciliata induced to ovulate with Ovaprim (●= first stripping; ○= second stripping) and human chorionic gonadotropin (□). Figures A and B give results for experiments 1 and 2, respectively.

CHAPTER 10

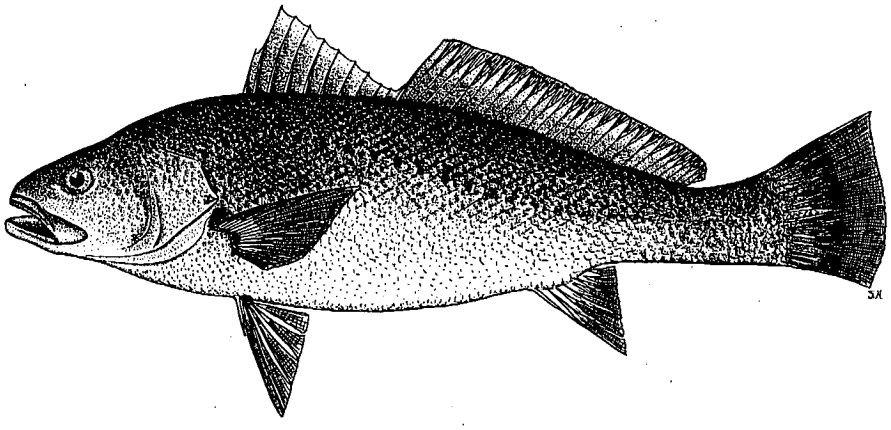
Swim bladder inflation in larvae of cultured sand whiting, *Sillago ciliata* Cuvier (Sillaginidae)

Stephen C Battaglene, Shannon McBride and R Bill Talbot

t is the published article: Battaglene, S. C., cBride, S., Talbot, R. B., 1994. Swim bladder inflation in larvae of cultured sand whiting, Sillago ciliata Cuvier (Sillaginidae), Aquaculture, 128(1-2), 177-192

Published in Aquaculture

PLATE 4 Mulloway, *Argyrosomus hololepidotus*
(Drawn by Jack Hannon)



CHAPTER 11

Hormone induction and larval rearing of mulloway, *Argyrosomus hololepidotus* (Pisces: Sciaenidae)

Stephen C Battaglene and R Bill Talbot

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t is the published article: Battaglene, S. C., Talbot, R.B., 1994. Hormone induction and larval rearing of mulloway Argyrosomus hololepidotus (Pisces: Sciaenidae), Aquaculture, 126(1-2), 73-81

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CHAPTER 12

General discussion

1. INTRODUCTION

The development of new marine fin-fish farming industries and enhancement programs in Australia will be dependent on a reliable supply of juvenile 'seed' fish. It is highly unlikely that these seed fish will be harvested from the wild (Chapter 1). Control of reproduction is therefore essential to the successful development of new marine fin-fish farming industries. However, many marine fish species will not breed in captivity without hormone treatment and the larvae are small and often difficult to rear. Intensively cultured larvae are particularly vulnerable during the transfer from endogenous to exogenous feeding. One of the important developmental features occurring at this time is initial swim bladder inflation.

The aim of my thesis was to select native marine fish with aquaculture potential from the temperate waters of New South Wales and to develop techniques for the large-scale breeding of four of these species. Manuscripts arising from this thesis provide the first published account of the hormone induced ovulation and larval rearing to metamorphosis of Australian bass *Macquaria novemaculeata* (Percichthyidae), snapper *Pagrus auratus* (Sparidae), mulloway *Argyrosomus hololepidotus* (Sciaenidae) and sand whiting *Sillago ciliata* (Silliganidae) in Australia. Hormone induction trials were conducted with mature fish collected from the wild. These fish were then retained in captivity and further hormone induction trials were conducted in following years. Larvae were reared intensively through to metamorphosis using live food and laboratory experiments were conducted to determine factors influencing survival, growth and initial swim bladder inflation.

2. REPRODUCTION

As in most vertebrates, reproduction in fish is controlled by the endocrine system comprising the hypothalamus, pituitary and gonads: the hypothalamic-pituitary-gonad axis (Liley and Stacey 1983). Fish receive environmental cues through their sensory organs which are relayed to the brain at the hypothalmic level, stimulating the production of gonadotropin releasing hormones. Gonadotropin releasing hormone then stimulates the pituitary gland to release gonadotropins (GtH) (Donaldson and Hunter 1983; Shelton 1989). Gonadotropins are hormonal glycoproteins of pituitary or placental origin which stimulate gonadal development (Donaldson and Hunter 1983). Recent research indicates there are two types of GtH, although the exact nature and function of the two types is unclear. It appears one (GtH I) is involved in gonad growth and the other (GtH II) in final gonad maturation (Kawauchi et al. 1989). In one of the few studies on marine fish, Tanaka et al. (1991) have purified two gonadotropic glycoproteins from the pituitaries of red sea bream *P. auratus*. The diurnal rhythm of GtH II secretion in *P. auratus* was correlated with oocyte maturation suggesting it is the maturational GtH (Tanaka et al. 1991). The term GtH is used in the following discussion to describe both gonadotropins, for simplicity , and because most studies have not distinguished between the two types.

There are many factors, both exogenous and endogenous, that influence gonad development during the entire cycle from primary gametogenesis through to final spawning (Fig. 1). Temperature and photoperiod are generally (separately or in combination) the primary exogenous influences which stimulate gonad growth and regression in temperate fish species (Munro and Lam 1990). They can also synchronise final maturation, ovulation and spermiation, although other more specific cues, eg. lunar periodicity, pheromones, and salinity, may also operate (Munro and Lam 1990).

It is now well established that many commercially important fish do not spawn spontaneously in captivity (Zohar 1988). In particular, females held in captivity often reach the final stages of vitellogenesis and then undergo rapid atresia (Zohar 1988). In the current study , final oocyte maturation, ovulation and spawning did not occur in captive or wild-caught fish of all four species without the application of hormones. The only exceptions were for *P. auratus* (Chapter 8) and *S. ciliata* (Chapter 9) that had already started ovulation when collected from the wild. According to Donaldson and Hunter (1983), there are two main reasons for the failure of sexually mature females to spawn naturally. First, inappropriate environmental conditions may not provide the correct spawning stimuli. Second, a generalised stress response can inhibit or disable reproduction. Inappropriate environmental conditions, eg. temperature and salinity, may be involved with fish held in the research pool at the Fisheries Research Institute at Cronulla and the broodstock ponds at Port Stephens. Stress effects, particularly handling, are probably also involved, particularly with wild-caught fish or those held in smaller tanks. *Pagrus auratus* appear particularly susceptible to stress (Carragher and Pankhurst 1991; Pankurst and Sharples 1992).The role of stress in the inhibition of reproduction in *P. auratus* is discussed in more detail in Chapter 8.

2.1 Induced Maturation, Ovulation and Spawning

The sequential nature of the hypothalamic-pituitary-gonad axis allows for intervention at several levels to promote gonad maturation (Donaldson and Hunter 1983; Fig. 1). Three hormones were used in the current study to induce final maturation, ovulation and in some cases spawning (Table 1). The most frequently used was human chorionic gonadotropin (hCG) a mammalian gonadotropin of placental origin, followed by Ovaprim, a proprietary mixture of luteinising hormone-releasing hormone analogue (LHRHa) and domperidone a dopamine antagonist, and finally LHRHa cholesterol based pellets.

HCG acts on the gonad by providing an artificial surge in gonadotropin which mimics the natural release of GtH from the pituitary. HCG is commonly used to induce ovulation in fish because it is highly effective for a wide range of freshwater and marine fish species, is uniform and standardised, relatively cheap, easily available, and can be stored for a long period (Donaldson and Hunter 1983; Rowland 1983; Shelton 1989). It proved effective at inducing ovulation in *M. novemaculeata* (Chapter 2) and *A. hololepidotus* (Chapter 11; Appendix A),

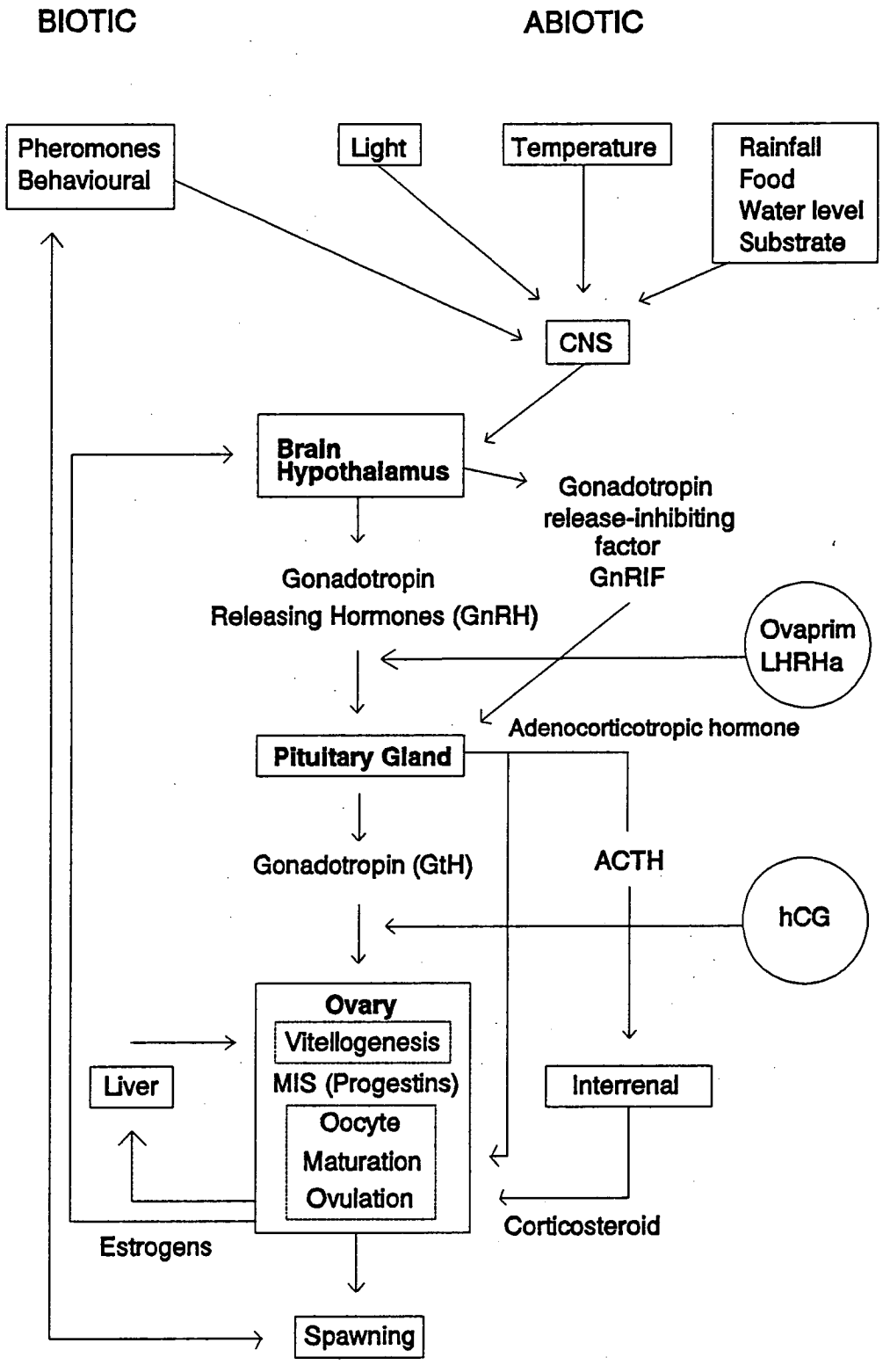

Figure 1 Diagram showing the relationship between factors influencing the hypothalamic - pituitary - gonad axis. Levels of intervention are given in circles. For a description of abbreviations see text. (Adapted from Shelton 1989).

TABLE 1 Comparison of the hormone-induced ovulation of four species of Australian marine fish. Details include the breeding season, method of collection, number of fish treated and hormones used.

Species	*M. novemaculeata*		*P. auratus*		*S. ciliata*		*A. hololepidotus*	
Source	Wild	Captive	Wild	Captive	Wild	Captive	Wild	Captive
Season, method and no.								
Start of breeding season	May	June	August	September	November	February	January	February
Finish of breeding season	August	September	December	October	March	Unknown	March	Unknown
Length of season (months)	3.5	4	4.5	2	4.5	1	3	1
Temperature range (oC)	13-18	10-22	17.8-21.3	12-26	20-23	23	20-22	22-24
Rod and reel	yes	yes	yes	yes	yes	no	yes	no
Long line	no	no	yes	no	no	no	no	no
Gill net	yes	no	no	no	yes	no	no	no
Trap	no	no	yes	no	no	no	no	no
Seine net	no	no	no	yes	yes	no	no	yes
Drain harvest	na	yes	na	yes	na	yes	na	yes
Males	>200	>200	94	137	43	3	3	24
Females	>200	190	81	111	67	3	3	12
Treated females	123	158	49	25	60	3	3	4
Running ripe females (%)	0	0	18.4	0	6.7	0	0	0
Mortality (%)	3.3	1.3	8.2	0	10.9	0	0	0
Type of spawner	Group synchronous		Asynchronous		Asynchronous		Group synchronous	
Hormones evaluated								
HCG (U/kg)	0 to 4000	0 to 1000	500 to 1000	1000	300 to 1000	no	1000	1000
Ovaprim (0.5ml/kg)	no	no	yes	yes	yes	yes	no	no
LHRHa (pellets 100ug/kg)	no	no	yes	yes	no	no	no	no
Preferred treatment	500 hCG	500 hCG	1000 hCG	LHRHa	Ovaprim	Ovaprim	na	na
Comparison 1000 U/kg hCG								
Number females treated	51	51	33	9	23	na	3	4
Ovulation (%)	62.7	76.5	68.8	33.3	73.9	na	100	100
Spawning (%)	35.3	60.8	0	0	0	na	0	0
Stripping (%)	27.5	15.7	100	100	73.9	na	100	100
Latent period (h)	34.1	39.3	16 to 100	79 to 92	33 to 44	na	32 to 36	32 to 44
Mean fertilisation (%)	72.0	73.0	46.8	44.0	56.6	na	17.7	20.3
Mean no. eggs/female	457264	395167	69 094	50600	46016	na	976920	914438

and to a lesser extent *P. auratus* (Chapter 6-8), and *S. ciliata* (Chapter 9), but was far less effective at inducing spawning. HCG proved ineffective at inducing ovulation in *M. novemaculeata* with oocytes < 860µm, *P. auratus* oocytes< 550µm, and *S. ciliata* oocytes <390µm (Table 1).

Ovaprim and LHRHa, operate at a higher level in the hypothalamic-pituitary-gonad axis than hCG (Fig.1), and stimulate the production and or release of the fishes own GtH (Donaldson and Hunter 1983; Zohar 1988). These gonadotropin releasing hormones were used on *P. auratus* and *S. ciliata* in an attempt to increase the proportion of females which ovulated and spawned. The use of Ovaprim increased the number of batches of ovulated eggs and latent period in *P. auratus* but did not lead to an increase in the number of eggs produced or improve fertilisation (Chapter 7). However, Ovaprim was a more reliable hormone than hCG for inducing ovulation in *S. ciliata* (Chapter 9). LHRHa pellets were only used on one occasion with captive *P. auratus* following unsuccessful attempts to induce ovulation using hCG and Ovaprim. The slow release pellets proved effective at stimulating both maturation and ovulation but not spawning (Chapter 8).

The important difference between LHRHa and Ovaprim, besides the obvious difference in delivery, is the inclusion of domperidone in Ovaprim. Dopamine is believed to be a gonadotropin release-inhibitory factor (GnRIF) in ostariophysan (Chang and Peter 1983; Donaldson and Hunter 1983).The domperidone present in Ovaprim is a dopamine antagonist and has been successfully used in combination with LHRHa to induce ovulation in cyprinids (Peter et al. 1988). However, the usefulness of dopamine antagonists in inducing maturation in other teleosts is less clear. The role of dopaminergic systems appears to be of minor importance in the regulation of GtH secretion in the few marine species studied and it is even possible that domperidone has a negative influence on GtH secretion (N. Pankhurst personal communication, 1995). Further controlled laboratory trials are required to determine if single injections of LHRHa are more effective than Ovaprim.

The reasons why fish fail to ovulate are complex. Although gametogenesis is clearly under the control of GtH, final maturation, ovulation and spawning are mediated by other hormones (Shelton 1989; Fig.1). These hormones include the prostaglandins and progesterones, in particular 17α,20β-dihydroxy-4pregnen-3-one (17,20βP), which is thought to be the maturation-inducing-steroid (MIS) in most teleosts (Scott and Canario 1987). Research to identify the MIS in *P. auratus* (Adachi et al. 1988; Ventling and Pankhurst 1995) and *S. ciliata* (Matsuyama et al. 1990) has been carried out and the MIS appears to be 17,20βP, although further studies are required to test the potency of other progestogens (Pankhurst and Carragher 1992).

The complex interaction of hormones influencing spawning is poorly understood.

Neurohypophysial hormones have been implicated in the short-term control of the spawning act in teleosts through stimulation of the oviduct and ovarian smooth muscle (Liley and Stacey 1983). Spawning as distinct from ovulation is a behavioural event mediated by physical and endocrine influences (Liley and Stacey 1983; Shelton 1989). Studies on birds and fish indicate that hormones affect behaviour and behaviour affects the endocrine system (Fenner 1992). Clearly the presence of a spermiating male is the most fundamental requirement for successful spawning, although females of some species will release eggs without the presence of males (Chapter 2). The physical influence of 'natural' conditions such as water temperature and depth, lighting and tank size may also play an important role in stimulating spawning. *Pagrus auratus*, for example, will spawn in sea cages and large public aquariums (Smith 1986; Foscarini 1988) but have not yet spawned in the research pool at the Fisheries Research Institute (Chapter 8). Controlled experiments to investigate spawning stimuli were not conducted during the current study. The influence of tank size (70 or 1000 l) on ovulation was found to be non-significant for *S. ciliata* (Chapter 9).

Fish which ovulate but do not spawn have to have their gametes stripped, necessitating additional handling and stress. Stripped fish usually produced fewer fertilised eggs than those resulting from spontaneous spawnings (Foscarini 1988; Chapter 2). One of the most significant problems encountered in the hormone induction trials in the current study was knowing when fish had ovulated so that they could be stripped. It was particularly difficult to determine when to strip *P. auratus*, and to a lesser extent *S. ciliata*, because they appear to ovulate on a daily cycle and they did not respond well to handling stress (Chapters 8 and 9). The viability of ovulated eggs retained in the ovary decreases rapidly in many warmwater species, a condition which is often termed 'overripening' (Bromage et al. 1994; Chapters 2, 8 and 9). Conversely, eggs can be stripped too close to ovulation (Bromage et al.1994), although 'underripening' appears to less of a problem. Overripening is defined as the aging process that occurs in an egg in the period following ovulation up to fertilisation and has clearly been shown to influence egg quality in salmonids and a number of marine species (Kjorsvik et al. 1990; Bromage et al. 1991, 1994). In fact, Bromage et al. (1994) considers that "overripening is a significant determinant of egg quality for many if not all fish".

One of the problems in determining stripping times was the difference in latency periods and fertilisation rates observed with *P. auratus* and *S. ciliata* collected using different methods or on separate occasions (Chapters 8 and 9).The optimum time to strip fish for maximum fertilisation is species-specific and temperature dependent and has been determined for relatively few species (Bromage et al. 1994). There are even fewer comparative studies on the same species, and where comparable studies have been conducted the results have sometimes given different developmental profiles, highlighting the difficulty of accurately pinpointing the time of ovulation (Holmefjord1991; Bromage et al.1994). More detailed studies are required to determine the timing of ovulation and optimal stripping times in *P. auratus* and

S. ciliata .The confounding influence of hormone-induced ovulation on egg quality also needs to be assessed.

It is generally easier to get male fish to undergo spermatogenesis and spermiation than it is to induce female fish to undergo vitellogenesis and ovulation (Donaldson and Hunter 1983). Spermatogenesis was completed in both wild and captive males in all four species in the current study, males often spontaneously spermiating on capture. A single dose of hCG, usually lower than that required for females, was often sufficient to increase milt quality and volume (Chapters 2 ,8 and 9). The administration of hCG is believed to mimic the natural rise in GtH that is associated with milt production (Pankhurst 1994). However, a detailed assessment of the effect of hormones on milt production was beyond the scope of the current study. Further information on the effect of hCG on milt production and quality is available for New Zealand *P. auratus* (Pankhurst 1994) and *S. ciliata* (Goodall et al. 1989).

The availability of spermiating males from the wild was generally greater than that of vitellogenic females for all species. Males also matured at a smaller size than females in all four species collected from the wild. There were occasional problems in the collection of males and poor milt volumes, particularly towards the end of the breeding season. Cryopreservation of sperm is a quick and efficient method of overcoming temporary shortfalls in supply and has been successfully used with *P. auratus* in Japan (Kurokura et al. 1986), *S. ciliata* (Young et al. 1992) and *A. hololepidotus* in Australia (S. Battaglene unpublished data, 1994).

2.2 Spawning Season and Source of Broodstock

The results of the current study suggest that *M. novemaculeata* and *A. hololepidotus* are group synchronous spawners in which maturation, ovulation and spawning generally occur once during the breeding season (Chapters 2 and 11). Whereas *P. auratus* and *S. ciliata* were found to be asynchronous or multiple spawners probably spawning at 24 h intervals for periods of up to three months (Chapters 8 and 9). Basically, these results obtained from hormone treated fish agree with the published biological information available on the four species. The exception is *M. novemaculeata*, which had been reported to be a serial spawner on the basis of oocyte size-frequency distributions and a detailed histological study (Harris 1986). However, in the absence of direct observations on spawning females in the wild, it is difficult to predict the number of spawnings and the number of eggs spawned from oocyte size-frequency distributions alone (de Vlaming 1983; West 1990). Other researchers have considered *M. novemaculeata* to be group synchronous spawners (C. Chamberlain unpublished data, 1989)

Macquaria novemaculeata proved to be the easiest fish to collect from the wild and to hold in captivity, followed in order by, *S. ciliata* , *P. auratus* and *A. hololepidotus* . According to Harris

(1986), *M. novemaculeata* spawn from late June to early September in the wild and the comprehensive hormone induction trials undertaken in Chapter 2 show that they can be induced to spawn for 3.5 months. Successful hormone treatment therefore precedes the natural spawning season and ceases prior to the end of spawning in the wild. This was a general trend with all four species (Table 1). The use of captive *M. novemaculeata* increased the breeding season and a combination of wild and captive broodstock was recommended (Chapter 2).

Pagrus auratus were induced to ovulate from August to December (Table 1). However, only females line-caught early in the season, presumably before they started ovulation and spawning, produced large numbers of fertilised eggs. The availability of vitellogenic female *P. auratus* depended on water temperatures (Chapters 6 to 8) which fluctuated according to the East Australian Current (Cresswell 1994). Variations in sea conditions were unpredictable, strong currents made trapping difficult and, combined with the problems of stress-induced atresia (Chapter 8), made the use of wild-caught *P. auratus* undesirable in the long term. The use of line-caught fish partially overcame the problem of stress but similar problems with weather conditions applied with long-lining. The long-term solution to the problems encountered in the procurement of *P. auratus* eggs appears to be domestication of broodstock and environmentally induced spawning in captivity (Chapter 6) as is currently practised in Japan with *P. auratus* (Foscarini 1988; Fukusho et al. 1986). F1 generation *P. auratus* and *A. hololepidotus* are currently being held in sea cages at the Fisheries Research Institute for this purpose.

The breeding season for *S. ciliata* was not ascertained in the current study, although mature fish are available for up to 5 months in Sydney (Burchmore et al. 1988). *Sillago ciliata* were readily available from the wild in large numbers at Port Stephens and proved easy to hold and matured in captivity (S. Battaglene unpublished data, 1994; Appendix A). *Sillago ciliata* as small as 215 g could be successfully induced to ovulate.

Argyrosomus hololepidotus proved the most difficult species to collect and very little is known about their reproductive biology outside that given in Chapter 10. Mature female *A. hololepidotus* are typically large and difficult to collect from shallow water. In contrast, mature females from off shore reefs appear to be smaller around 5 Kg (Appendix A). Fish captured at depth (>20 m) were particularly difficult to keep alive because of the damage sustained by the over -inflation of the swim bladder. The procedures used to deflate the swim bladder and handle the fish are described in Talbot and Battaglene (1993). To highlight the difficulties involved in obtaining broodstock, 19 fish were caught off reefs in January 1995, but only two small fish (<4kg) were successfully transported back to the hatchery. Furthermore, during the study only three female *A. hololepidotus* were successfully collected from the wild, transported and hormone-induced to ovulate. Four captive *A. hololepidotus* were also

successfully hormone-induced. The small number of fish treated (n=7), limited the amount of experimentation that could be conducted on *A. hololepidotus* and further research is required to determine the extent of the breeding season. The long-term solution to the supply of *A. hololepidotus* eggs is the same as that for *P. auratus*: captive stocks held in temperature and photoperiod controlled tanks (Chapter 11). *Argyrosomus hololepidotus* held in the research pool at the Fisheries Research Institute matured in captivity but those held in a 50 000 l tank at the Port Stephens Research Centre did not (Appendix A). Fish grew quickly at both locations but were particularly susceptible to external parasites and needed to be treated prophylactically with formalin and malachite green. They also ceased feeding when the water temperatures rose above 25 °C, suggesting they may be sensitive to high water temperatures.

It appears that the start of the breeding season for all four species can be brought forward by collecting fish from the northern distribution of their range. Likewise, the breeding season can be extended by collecting fish from the southern distribution of their range at the end of the season. For example, *P. auratus* spawn in the north from late May to August (Mc Nee et al. 1993), in Port Stephens from July to December (Chapter 8) and in southern Australia between late October and early March (Lenanton 1974). However, the extent to which this will be a practical consideration is debatable and care should be exercised in interbreeding fish from different stocks or breeding populations. Some research on stock discrimination has been carried out for *P. auratus* (MacDonald 1982; Francis and Winstanley 1989), *A. hololepidotus* (Black and Dixon 1992) and *S. ciliata* (Dixon et al. 1987). Unfortunately there is no information available on *M. novemaculeata* , which conceivably has a greater potential for stock discrimination because of its catadromous nature.

3. LARVICULTURE

During the current study larval mortality in all four species peaked during two key larval development phases: at first feeding and during weaning (Chapters 5, 6, 10 and 11). Early larval mortality is difficult to quantify but was generally higher than that which occurred at weaning. Early larval mortality often peaks during or soon after the transition from endogenous to exogenous feeding, leading to the 'critical period' concept (May 1974; Vladimirov 1975; Hunter 1976). However, there has been considerable debate about the relative role of first-feeding and the matching of suitable food in early larval survival. This is particularly true when extrapolating to natural populations where predation can be an important factor (Blaxter 1988; Leis 1991). Clearly, many other factors can influence larval survival besides food resources (Chapter 1). Blaxter (1988) listed five potentially critical periods through which larvae have to pass to allow development to proceed. They were: hatching, first -feeding, respiration, swim-up (initial swim bladder inflation) and metamorphosis. The first four critical periods occur early during larval rearing and are interrelated.

3.1 Initial Swim Bladder Inflation

The swim bladder develops as a dorsal or lateral diverticulum of the gut or oesophagus (Duwe 1955; Steen 1970; Schwarz 1971). The pneumatic duct connecting the swim bladder to the gut disappears after initial inflation in physoclists (Steen, 1970; Chatain and Ounais-Guschemann 1990). This condition is considered a phylogenetic repetition of the more primitive physostomous condition (Duwe 1955). In general, fish use two mechanisms to initially inflate the swim bladder (Lagler et al. 1962; Chatain and Ounais-Guschemann 1990). Initial inflation results from either internal gas production, usually from the rete mirabile or gas gland (Lagler et al. 1962; Steen 1970; Schwarz 1971), or by the larvae gulping air usually at the water surface (Hunter and Sanchez 1976). Chatain and Ounais-Guschemann (1990) pointed out that the mechanisms of initial inflation are complex and poorly understood. For example, internal gas production can occur via i) vacuolation of the glandular epithelium, ii) rete mirabile or gas gland, iii) degradation of organic material, or iv) digestion (Chatain and Ounais-Guschemann 1990).

Initial intake of an atmospheric gas via the pneumatic duct into the swim bladder lumen or "swim up" has been experimentally confirmed for physostome larvae eg. *Engraulis mordax* (Hunter and Sanchez 1976), and salmonids (Tait 1960), and physoclist larvae that possess a functional pneumatic duct in the early larval stages eg. *Morone saxatilis* (Doroshev and Cornacchia 1979), *P. auratus* (Kitajima et al. 1981, 1994), *Sparus auratus* (Chatain and Ounais-Guschemann, 1990). However, the relative contribution of internal gas production to initial inflation in these species has not been studied. Once the pneumatic duct in physoclistous fish closes, gas regulation is usually by the gas gland (Steen 1970).

Lumenal dilation of the swim bladder with liquid may precede initial inflation in some species (Chapter 3). Dilated (liquid filled) swim bladders were observed in all four species examined in this thesis. However, it appeared that dilation was typically a malfunctioning of the swim bladder in which the pneumatic duct passed sea water into the swim bladder (Chapter 3). In a few isolated cases this was confirmed by the presence of rotifers in the swim bladder of *M. novemaculeata* and *P. auratus.* The malfunction condition has also been described for cultured *P. auratus* and *Plecoglossus altivelis* in Japan (Ochiai et al. 1977; Takashima et al. 1980). Interestingly, initial inflation in *S. ciliata* was not preceded by dilation, but dilated swim bladders were present in older larvae with functioning swim bladders (Chapter 10). The early development of the swim bladder has been described histologically for: *Lepomis cyanellus* (Duwe 1955), *Percina caprodes* (Grizzle and Curd 1978), *Melanogrammus aeglefinus* (Schwarz 1970), *Morone saxatilis* (Doroshev and Cornacchia 1979; Bennett et al. 1987), *P. auratus* (Yamashita 1982), *Solea solea* (Boulhic and Gabaudan 1992), *Dicentrarchus labrax* and *S. auratus* (Soares et al. 1994).

The failure of the swim bladder to inflate or swim bladder dysfunction in larval marine fish was first recorded over 20 years ago and has now been recorded in over 25 species of cultivated larvae belonging to 13 families(Table 2; Appendix B). Clearly, it is a common problem in the intensive culture of many commercially important fish, particularly species belonging to the Moronidae, Sparidae, Sciaenidae and related families (Table 2). The number of species in which swim bladder dysfunction is a problem is increasing as more species are cultivated and closer examination of factors influencing larval development and survival occurs (Table 3).

Swim bladder dysfunction is restricted to intensively reared marine fish and a few freshwater fish with 'marine type larvae'.Typically marine eggs are single, buoyant, and with a modal diameter of about 1mm (Blaxter 1988). They consequently produce small larvae that are often poorly developed, lacking mouth, pigmented eyes and digestive systems (Blaxter 1988). There are relatively few species for which the mechanism, and optimal conditions for initial swim bladder inflation are known. Four marine species that have received detailed study are, *M. saxatilis*, *P. auratus*, *S. auratus*,and *D. labrax.* One freshwater species, *Stizostedion vitreum* has also been well studied (Tables 2 and 3). However, even for these species there have been few controlled experiments to detect the interactions of biotic and abiotic factors.

Failure of the swim bladder to inflate can cause death (Spectorova and Doroshev 1976), and reduced growth of larvae (Chatain 1987), and severe skeletal deformities in surviving adults (Paperna 1978; Chatain and Dewavrin 1989; Kitajima et al. 1994). Larvae without functional swim bladders swim continuously in a head up position to avoid sinking. This oblique swimming position puts pressure on the backbone and results in lordosis in surviving adults (Chatain 1994; Kitajima et al. 1994). Larvae without functional swim bladders are usually smaller, darker and less conditioned than larvae with swim bladders. They are consequently more susceptible to stress induced mortality (Chatain 1986,1987; Chapman et al. 1988a). The reduced growth of cultured larvae without swim bladders is a result of the energy expended to remain in the water column possibly combined with a reduced feeding capacity. Larvae without swim bladders at metamorphosis were about 25% smaller for *M. novemaculeata* (S. Battaglene unpublished data, 1989), 20% for *P. auratus* (Chapter 6) and 12% for *A. hololepidotus* (Chapter 11).

Swim bladder development usually occurs just before or at the time of first feeding and the assimilation of the yolk-sac (Table 2). It is therefore difficult to separate the relative contribution of swim bladder dysfunction, and the failure to start exogenous feeding, to mortality. To complicate matters further, the endogenous production of some important hormones, eg. thyroxine and triiodothyronine also occur at this time and can influence larval survival (Lam 1994). The duration of the yolk-sac period varies according to species, temperature and egg size (Blaxter 1988; Table 2). Cultured species in which swim bladder dysfunction is a problem are generally small. Larvae are typically around 3 to 5 mm at the

TABLE 2 Details of developmental timing of initial swim bladder inflation in cultured fish larvae, including the earliest recording and number of scientific publications for each species.

Family	Species	Earliest record	Number of publications	Temperature °C	Initial inflation		Absorption at inflation		Diel rhythm
					Age (days)	TI (mm)	Yolk	Oil	
Moronidae	*Dicentrarchus labrax*	1986	10		5.5 to 6.5	5 to 7	yes	yes	yes
	Morone saxatilis	1970	12	18	5 to 7	7.5	yes	no	
	M. chrysops x M. saxatilis	1988			4				
Percichthyidae	*Macquaria novemaculeata*	1989	4	19	6 to 11	4.5	yes	no	
Serranidae	*Lateolabrax japonics*	1984	2	16	10 to 18	5.5 to 7.5			
Sparidae	*Sparus auratus*	1986	10		4 to 5	4 to 5	yes	yes	
	Acanthopagrus cuvieri	1983	2		4	4 to 5			
	Pagrus major =(P. auratus)	1977	16		5 to 10	3.5 to 4	yes	yes	yes
Centrarchidae	*Micropterus salmoides*	1953	1		2 to 4	4 to 5			
Mugilidae	*Mugil cephalus*	1975	4	19 to 24	5 to 7	3 to 3.5	yes	no	
Cichlidae	*Sarotherodon mossambica*	1979	2	25.5	7 to 9	9 to 10.5	no		
	Hemichromis bimaculata	1940	1		3 to 4				
Carangidae	*Seriola aureovittata*	1994	1		5 to 10	5.5 to 6			
Gadidae	*Boreogadus saida*	1975	1	1.5	6 to 7	6.5 to 7.2	no	no	
Latrididae	*Latris lineata*	1991	1	14 to 16	12	5.9 to 6.2	yes	yes	no
Clupeidae	*Brevoortia tyrannus*	1989	3	20		>10			yes
	Engraulis mordax	1976	1	16.5		8.5			yes
Percidae	*Stizostedion vitreum*	1986	8	15	7 to 14				
Sciaenidae	*Argyrosomus hololepidotus*	1994	1		4 to 11	3 to 3.7	yes	no	
	Sciaenops ocellata	1981	1	25	4	3 to	yes		
	Atractoscion nobilis	1989	1	18.5	4 to 6	3.5	yes	yes	
Sillaginidae	*Sillago ciliata*	1994	1	24	4 to 5	2.8	yes	yes	yes
Soleidae	*Solea solea*	1992	1	18-20	10	4.5	no	no	
Scopthalmidae	*Scophthalmus maeoticus*	1976	2	18	6 to 7	4			
Acipenseridae	*Huso huso*	1983	1			80-140			

time of inflation (Tables 2 and 4), and start inflation and feeding within one week of hatching. They consequently have small endogenous reserves. The four species examined in the current study follow this pattern (Table 4). In most physoclist fish the pneumatic duct appears to close within one week of initial inflation (Table 2). However, the exact timing of inflation, as found for *M. novemaculeata* in Chapter 3, and the closing of the pneumatic duct has not been documented for many species.

Histological studies of *M. saxatilis* and *P. auratus* suggested that failure of the swim bladder to inflate is irreversible once the pneumatic duct closes (Kitajima et al. 1981; Bennett et al. 1987). In contrast, late swim bladder inflation has been recorded for *P. auratus*, when fish are 1-8g (Chatain 1982), and *S. auratus* at 7-54g (Chatain 1994). The mechanisms involved in late inflation are unknown but must involve internal secretion of gas (Chatain 1994). Late inflation may occur in other species but has not been recorded because larvae without functional swim bladders are cannibalised or die before late inflation can occur. For example, larval *P. auratus* without functional swim bladders died at weaning (Chapter 6) and *A. hololepidotus* without swim bladders were cannibalised (Chapter 11). Late inflation is therefore not a viable alternative to initial inflation, particularly as spinal lordosis develops in larvae without swim bladders and is generally not corrected following late inflation (Chatain 1994). Fish without swim bladders are usually removed during grading sometimes using anaesthetic and or changes in salinity (Chapman et al. 1988b; Chatain and Corrao 1992; Barrows et al. 1993a).

In some species initial swim bladder inflation takes place at night (Chapter 10). This is presumably a behavioural trait to avoid predation and many larvae are negatively phototaxic. Several species of physostomous larvae inflate their swim bladders at night and deflate them during the day (Uotani 1973; Hunter and Sanchez 1976; Hoss et al. 1989). Similarly, some physoclistous species including *P. auratus*, regulate the volume of gas in the swim bladder on a diel cycle partly deflating the swim bladder during daylight (Kitajima et al. 1985; Steffe 1991; Kitajima et al.1993). The diel pattern of nocturnal inflation was particularly evident in *S. ciliata*. I proposed that it was a strategy to conserve energy at night when larvae were not searching for food (Chapter 10).

Species-specific abiotic and biotic factors can influence initial swim bladder inflation in cultured fish (Hadley et al. 1987; Chatain and Corrao 1992). Abiotic factors of interest include: surface films, water quality, water turbulence, aeration, light, temperature and salinity. Biotic factors influencing inflation include: parental stock, acclimation history, and diet. Interpretation of the relative role of environmental effects on initial swim bladder inflation is complicated by the interactions between abiotic factors and biotic factors.

The presence of an 'oily' surface film is the single most commonly reported cause of swim bladder dysfunction in intensively reared larvae (Table 3). Oily films block access to the water surface and prevent larvae from inflating their swim bladder (Colesante et al. 1986; Foscarini 1988; Chatain and Ounais-Guschemann 1990; Chapter 10). For example, the production of oily surface films preventing larvae gulping air is the major cause of swim bladder dysfunction in *S. vitreum* (Colesante et al. 1986), *P. auratus* (Foscarini 1988; Chapter 6), *S. auratus,* and *D. labrax* (Chatain and Ounais-Guschemann 1990).

Oily surface films are typically produced by the end-products of hatching eggs, dead eggs and larvae, nutritionally enriched live feeds, or oily artificial feeds. I found that reductions in the production of surface films can be achieved in three ways:
i) by using separate incubation systems for eggs and selective stocking of good quality yolk-sac larvae (ie. those with good fertilisation and hatch rates).
ii) by delaying feeding till after swim bladder inflation.
iii) by avoiding oily enrichment products or artificial feeds during early rearing, or containing them to feeding rings.

If the production of oily films is unavoidable then they must be removed. Simple surface skimmers were developed and tested in France in the 1980's (Foscarini 1988; Chatain and Ounais-Guschemann 1990; Dewavrin and Chauveau 1990). Skimmers, now available in a multitude of variations depending on air supply and tank size (Dewavrin and Chauveau 1990), remove the film by gently blowing air across the water surface and collecting the resulting scum in a floating trap. Swim bladder inflation rates in *S. auratus* and *D. labrax,* increased from <50% to >80% when surface skimmers were used (Chatain and Ounais-Guschemann 1990). A similar level of improvement was achieved for *P. auratus* in the current study (Appendix C).

Skimmers need to be cleaned regularly and the air flow adjusted to avoid entrapping larvae (S. Battaglene unpublished data, 1994). Quantitative studies showing the effectiveness of surface skimmers have been restricted to *S. auratus* and *D. labrax* (Chatain and Ounais-Guschemann 1990). Surface skimmers are not always required. For example, excellent inflation rates (>90%) are achieved routinely with *M. novemaculeata* by avoiding the production of surface films and provision of suitable environmental conditions (Chapters 3 and 4; Appendix C). High mortality of *Latris lineata* larvae has occurred with the use of surface skimmers (L . Searle personal communication, 1993) and *S. ciliata* (Chapter 8) . The potential negative influence of surface skimmers in the intensive culture of very delicate larvae needs further research.

Only a few other methods of collecting, containing or dispersing surface films have been tested, including surface sprays, hydrojets, feeding rings and air fans (Kitajma et al. 1981;

TABLE 3 Summary of factors influencing initial swim bladder inflation in cultured marine fish larvae.

Family	Species	Factors influencing initial inflation							Reduced growth	Deform-ities	Sorting	Key reference
		Surface access	Aeration @ Turbulence ml/min	Salinity ‰	Light Lux	Temp. °C	Nutrition	Parental				
Moronidae	*D. labrax*	yes	40	10 to 20	70				23 to 33	lordosis	yes	Chatain 1989
	M. saxatilis	yes	yes	5		16		yes	yes		yes	Hadley et al. 1987
Percichthyidae	*M. novemaculeata*		<50	15 to 35	<1			yes	yes	lordosis	no	Battaglene and Talbot 1990
Serranidae	*L. japonics*		50 to 100							lordosis		Hayashida et al 1984
Sparidae	*S. auratus*	yes	yes		600		HUFA		23 to 33	lordosis	no	Chatain 1989
	A. cuvieri	yes	200			22 to 26				yes		Al-Abdul-Elah et al. 1983b
	A. schlegeli									lordosis		Kitajima 1979
	P. auratus	yes	50				HUFA	yes	yes	lordosis	yes	Foscarini 1988
Cichlidae	*S. mossambica*	no										Doroshev et al. 1981
Carangidae	*S. quinqueradiata*		50 to100							lordosis		Kitajima et al.1994
	S.aureovittata	yes					yes			lordosis		Kitajima et al.1994
Gadidae	*B. saida*											Aronovich et al. 1975
Latrididae	*L. lineata*				<200				yes			Ruwald et al. 1991
Clupeidae	*B. tyrannus*	yes			yes							Hoss et al. 1989
	B. patronus	yes			yes							Hoss and Phonlor 1984
	E. japonicus	yes			yes							Uotani 1973
	E. mordax	yes			yes							Hunter and Sanchez 1976
Percidae	*P. fluviatilis*			no	yes							Ribi 1992
	S. vitreum	yes				24			yes	yes	yes	Barrows et al. 1993
Sciaenidae	*A. hololepidotus*								yes	lordosis		Battaglene and Talbot 1994
Sillaginidae	*S. ciliata*				yes							Battaglene et al. 1994
Scopthalmidae	*S. maeoticus*											Spectorova and Doroshev 1976

TABLE 4 Comparison of the development and intensive larval rearing trials of four species of Australian marine fish. DAH = Days after hatch

Species	*M. novemaculeata*	*P. auratus*	*S. ciliata*	*A. hololepidotus*
Larval development				
Mean water temperature	17.6 to 21.6	18.8 to 23.9	21.6 to 24.5	22.6 to 24.0
Size of eggs (mm)	1.0-1.2	0.9-1.0	0.6-0.7	0.8
Size of oil globule (mm)	0.4	0.2	0.2	0.3
Hatching time (h)	48 at 20°C	28 at 22°C	24 at 25°C	29 at 25°C
Size at hatch (mm)	2.7 to 3.5	3.1	2.5(0.1)	2.3
Size 6 DAH	4.4	3.2	2.8	3.1
Size 19 DAH	5.4 to 6.2	5.6	6.9	6.3
Size 29 DAH	6.4 to 8.1	11.8	9.7	10.2
Swim bladder inflation (DAH)	5 to 11	7 to 11	4 to 5	3 to 4
Mouth open (DAH)	3 to 4	3	3	2
Start feeding (DAH)	5 to 7	6	4	4
Yolk-sac absorbed (DAH)	7	3	3	3
Caudal bending (DAH)	23	19	19	12
Metamorphosis (DAH)	39	25	21	34
Metamorphosis (mm)	9.7	8.6(0.5)	8.1	12.1
Scale formation (mm)	16 to 24	9.1-13.7	UN	15-26
Larval rearing				
No.trials	22	11	4	7
Mean length of trials (days)	10 to 40	19 to 75	20 to 50	25 to 51
Stocking density (larvae/L)	36 to 100	0.6 to 31	60 to100	5 to 50
Rotifers (DAH)	5 to 10	3 to 6	3 to 4	3 to 4
Brine shrimp (DAH)	7 to 19	18 to 25	18 to 26	12
Weaning (DAH)	NA	24 to 55	43	24 to 39
Start cannibalism (DAH)	NA	26	NA	22
Survival (%)	0 to 63	0.8 to 68	0 to 20	0 to 55

Barrows et al. 1988,1993b; Chatain and Ounais-Guschemann 1990). Surface sprays increased swim bladder inflation in *S. vitreum* (Barrows et al. 1993b; Moore et al. 1994) but not *S. auratus* and *D. labrax* (Chatain and Ounais-Guschemann 1990). Most methods proved inferior to surface skimmers because they induced turbulence in the upper surface layer preventing larvae from reaching the water surface.

Besides poorly designed surface cleaning methods, turbulence in larval rearing tanks can be created by water inflow and aeration. Aeration and related water agitation have affected initial inflation in cultured *M. novemaculeata*, *P. auratus*, *Acanthopagrus cuvieri*, *M. saxatilis* and *Seriola quinqueradiata* (Table 3; Chapters 3 and 4). Aeration also affects the survival of cultured larvae and optimum rates increase as larvae grow (Barahona-Fernandes 1978). The somewhat contradictory optimum aeration rates reported in the literature reflect the different swimming capacities of the larvae, the different size of rearing systems, differences in larval stocking densities, and the possible confounding influence of surface films in some studies.

Other factors such as live food distribution, accumulation of waste products and the maintenance of good water quality, need to be considered before optimal aeration and water inflow rates can be determined. Very few studies have determined optimal aeration rates in relation to swim bladder inflation (Chapter 3). A recent exception is a study by Kitajima et al. (1994) showing optimal inflation rates using 50 ml air/min/100 l for *P. auratus* and 50 to 100 ml/min/500 l for *Lateolabrax japonicus*. These rates are similar to those found experimentally for *M. novemaculeata* (Chapter 3). During the current study aeration rates of 50 to 150 ml/min/2000 l were used in the early stages of production trials with all four species. Further details on the influence of aeration on swim bladder inflation in *M. novemaculeata* are given in Chapters 3 and 4.

A further consideration in determining optimum aeration rates is the total amount of dissolved gas and the influence of gas pressure gradients on internal gas secretion. As previously mentioned, the relative importance of internal gas secretion in initial inflation in larvae that require surface access is unknown. However, experiments with *Sarotherodon mossambica* larvae, a physoclist that does not require surface access for initial inflation, showed a negative relationship between inflation and stocking density in closed (oil topped) flasks (Doroshev and Cornacchia 1979). Poor inflation was attributed to a reduction in dissolved gases (Doroshev and Cornacchia 1979). Similar observations were made for *S. ciliata* (Chapter 10).

The data presented in Chapter 3 provides the first detailed evidence of the inhibitory influence of light intensity on swim bladder inflation in cultured larvae. Larvae that are negatively phototactic, for example, *D. labrax, M. novemaculeata* and *P. auratus* are initially reared in the dark or under very low light intensities to promote swim bladder inflation (Foscarini 1988; Ounais-Guschemann 1989; Chapters 3 and 6). The importance of reducing light during the

early rearing of *D. labrax* to promote swim bladder inflation was the focus of research in France during the 1980's (Weppe and Joassard 1984; Anon 1987) but for commercial reasons details of this research have not been published (B. Chatain personal communication, 1991).

As outlined in Chapters 3 and 10 keeping larvae in the dark until the start of exogenous feeding has two advantages. First, it helps negatively phototactic larvae in reaching the water surface. Second, larvae expend less energy searching for food and avoiding predators. The energy conserved may be converted into growth. Larger larvae are presumably more capable of not only reaching the water surface to inflate their swim bladders, but of catching and eating larger prey. However, most fish larvae are visual feeders and light is required at first feeding (Blaxter 1986). Some intensively cultured larvae are found with food in the gut at night eg. *M. saxatilis* and *P. auratus,* although ingestion may be accidental due to high prey densities (McHugh and Heidinger 1977; P. Pankhurst 1994).

Optimal light intensities and photoperiods for growth and survival in young larvae vary greatly and are species-specific. Marine fish larvae have been successfully reared at light intensities of 1-10 000 Lux (Tucker 1992a; Huse 1994) . But high light intensities are lethal to many newly hatched larvae. Survival can be increased, in some species, by lowering light intensity during the early stages of rearing (Nash and Kuo 1975; Kraul 1983; Johnson and Katavic 1984; Huse 1994). For example, maximum feeding in *Gadus morhua* occurs at 1 Lux and for *Pleuronectes platessa* larvae at 87 Lux (Huse 1994). Alternatively, light levels of 600-1500 Lux are reported to increase survival of intensively reared *Scophthalmus maximus* (Huse 1994), *S. auratus* (Ounais-Guschemann 1989; Tandler 1993) and *S. ciliata* (Chapter 8). Continuous light can increase growth of some cultured larvae but often results in increased mortality (Barahona-Fernandes 1979; Tandler and Helps 1985). Further detail on the influence of light on swim bladder inflation in *M. novemaculeata* is given in Chapter 3 and on *S. ciliata* in Chapter 10.

The eggs and larvae of many marine species are euryhaline and eurythermic, adaptations that enable them to inhabit estuarine environments. The tolerance of larvae to combinations of temperature and salinity are species specific and change with age, initial egg quality and acclimation of broodstock (Blaxter 1969; Holliday 1969; Alderdice 1972, 1988). The effect of temperature on hatching success and larval development is generally greater than that of salinity (Blaxter 1969; Holliday 1969). However, temperature and salinity tolerance of eggs and larval fish should be studied in combination because of their interactive effects on osmoregulation (Alderdice and Velsen 1971; Alderdice 1972, 1988; Marangos et al. 1986).

The effect of salinity on initial swim bladder inflation has been better researched than that of temperature, reflecting the obvious theoretical link between water density and larval buoyancy

(Hadley et al. 1987). However, initial inflation has not been found to be greatly affected by changes in salinity for *M. saxatilis* or *M. novemaculeata*. Chapman et al. (1988a) found that increasing salinity from 0 to 6.5 or 10‰ did not influence inflation in *M. saxatilis*. Similarly, Hadley et al. (1987) found that salinity (2.5 or 7.5‰) influenced survival but not inflation in *M. saxatilis*. However, when *M. saxatilis* larvae were exposed to salinities higher than 7.5‰ inflation was greatly suppressed or lacking (Cornacchia 1982, reported in Hadley et al. 1987). Initial inflation and survival was high and not significantly different for *M.novemaculeata* over a salinity range of 15 to 35‰ but the number of viable larvae greatly decreased below 10‰ (Chapters 3 and 4). There was also a tendency for increased inflation in *M.novemaculeata* at higher salinities in combination with high aeration.

The influence of salinity on initial inflation in *D. labrax* is more complicated. Highest inflation rates were reportedly obtained by lowering salinity from 35‰ to 10-20‰ by day 4 after hatch, keeping them constant to day 10, and then increasing them over the latter period of rearing (Barnabe and Guissi 1993). However, there was a significant interaction between salinity and dietary factors (Barnabe and Guissi 1993). Tandler (1993) reported increased inflation rates for *D. labrax* and *S. auratus* reared at 25‰ versus 40‰. Swim bladder inflation rates for *D. labrax* and *S. auratus* reared at 25‰ were 75% and 93%, respectively. High salinities (36‰) have also been implicated in hypertrophy of the swim bladder in *D. labrax* (Johnson and Katavic 1984; Barnabe and Guissi 1993).

The effects of temperature on larval survival and development has been documented for *S. auratus* (Tandler et al. 1989a), *D. labrax* (Marangos et al. 1986), *M. saxatilis* (Kellogg et al. 1984), and *M. novemaculeata* (van der Wal 1985), all species that exhibit swim bladder dysfunction. However, the effect of varying temperature on swim bladder inflation has received almost no study. Hadley et al. (1987) found that at 19°C, a temperature considered close to optimum for *M. saxatilis*, inflation and survival was reduced compared with that at 16°C. This may be due to the effect of temperature on endogenous hormone production (see below). Further study is required into the effects of temperature on swim bladder inflation.

Biotic factors influencing initial swim bladder inflation have also received very little study and typically play a more secondary role. However, they are very important for two reasons. First, they may explain why even after determination of optimal rearing conditions some larvae (typically 25%) still fail to inflate their swim bladders (Appendix C). Second, understanding the role of biotic factors may help in reducing the variability that exists between replicates in larval rearing experiments designed to test the effect of abiotic factors. Studies have suggested that variation in inflation rates may be related to genetic factors (Paperna 1978; Taniguchi et al. 1984), dietary factors (Kitajima et al. 1981,1994; Al-Abdul-Elah et al.1983b; Barnabe and Guissi 1993), and spawning stocks and procedures (Hadley et al. 1987; Chapman et al.1988a).

The influence of larval nutrition on swim bladder inflation is unclear but probably plays a secondary role (Koven et al. 1990). Some studies have tried to link swim bladder dysfunction with poor broodstock or larval nutrition. Harel et al. (1992) suggested that there was reduced egg viability, hatching and swim bladder inflation in *S. auratus* when n-3 highly unsaturated fatty acids (n-3 HUFA) dropped below 20 mg/g dry weight, but no data were provided. Kitajima et al. (1994) showed that increasing the amount of n-3 HUFA in rotifers fed to *P. auratus* and *Seriola aureovittata* also increased inflation. However, Koven et al. (1990) in a detailed study of *S. auratus* found no compelling evidence that HUFA affected larval survival or swim bladder development. In another study *D. labrax* reared on *artemia* that were low in HUFA actually had higher inflation rates than larvae reared on rotifers enriched with cod liver oil (Barnabe and Guissi 1993), although the confounding influence of surface films may explain this anomaly. The importance of HUFA in the diet of larvae is discussed in more detail in section 3.2.

A relationship between swim bladder dysfunction and the presence of calculi in the kidney and urinary bladder has been reported for *S. auratus, D.labrax* and *P. auratus*. (Ueda et al. 1970; Yamashita 1971; Modica et al. 1993; Berg et al. 1983),). Similar calculi were observed in all four species in the current study. However, there did not appear to be any relationship between the crystals and swim bladder dysfunction or larval mortality in the current study. For example, the calculi in the bladder of intensively reared *P. auratus* typically appeared in 20% of seven -day- old larvae increasing to 90% of 14 day-old-larvae irrespective of swim bladder function. The calculi persisted in all species until weaning, and possibly beyond, but became increasingly difficult to detect as the juveniles became fully scaled and pigmented. The causes of the condition are unknown and it is presently unclear if the presence of calculi is a pathological condition as suggested by Modica et al. (1993).

Recent evidence suggests that hormones are passed on to eggs by broodfish and that these can influence egg and larval quality (Lam 1994). Endogenous hormone production takes place in many species at the start of exogenous feeding. Factors influencing endogenous production of hormones (eg. thyroxinogenes) are poorly understood but presumably include temperature (Tanangonan et al. 1989; Lam 1994). Artificial elevation of thyroid hormones (triiodothyronie) in female *M. saxatilis* broodstock has been shown to increase swim bladder inflation and survival in larvae (Brown et al. 1988). Differences in maternal hormone levels among broodstock may therefore influence swim bladder inflation rates. Hadley et al. (1987) suggested that high variability of developmental characters, including inflation rates for *M. saxatilis*, was due to variation among progenies.

In conclusion, abiotic factors play a major role in initial swim bladder inflation of intensively cultured larvae, while biotic factors play an important secondary role. The interactions of

abiotic and biotic factors are also important. A conceptual framework for these interactions was provided by Hadley et al. (1987). They proposed a hypothetical model of factors affecting initial swim bladder inflation in *M. saxatilis*. In this model swim bladder dysfunction resulted from two primary sources. First, due to failures during organogenesis, caused by genetic factors, broodstock nutrition, hatchery procedures and incubation temperatures. Second, from adverse environmental influences during the swim bladder inflation phase. The results presented in my thesis support this model; a modified version of which is presented in Figure 2.

Even where optimal rearing conditions have been determined, a significant proportion of many intensively cultivated species are still being produced without functional swim bladders (Hadley et al. 1987; Chatain and Ounais-Guschemann 1990; Appendix C). Further improvements in inflation rates will probably come from an understanding of the role biotic factors play in the production of quality yolk-sac larvae. Factors influencing inflation are species -specific and should be determined experimentally for each new species. The results presented in this thesis indicate the following conditions should be used in the absence of species specific experimental data:

i) Broodstock should be maintained on a balanced diet high in HUFA.

ii) Eggs should be incubated and hatched in separate containers to those in which the larvae are reared.

iii) Surface skimmers should be used for the first two weeks of rearing, starting at feeding or from hatch if the water surface is oily.

iv) Larval feeding should be delayed until just before or preferably after initial swim bladder inflation.

v) Larvae should be kept in the dark until just before or if possible after initial swim bladder inflation.

vi) Low aeration or no aeration should be used to avoid turbulence.

vii) Light intensities should be chosen according to the distribution of larvae in the tank, the percentage of larvae feeding and the amount of feeding per individual.

vii) Light intensities and aeration should be adjusted as the larvae grow.

3.2 Larval Rearing Trials

There is increasing evidence that broodstock nutrition, and in particular the level of n-3 HUFA incorporated into eggs, is an important determinant of early larval size and survival (for reviews see Watanabe 1985; 1986; Tucker 1992b). Special care was taken in the current study to boost the nutritional profile of food given to broodstock held in captivity. Wherever possible fish were fed a wide range of food including pilchards, prawns, squid and vitamin supplements.

Both the rate of yolk absorption and the efficiency of yolk use are important determinants of

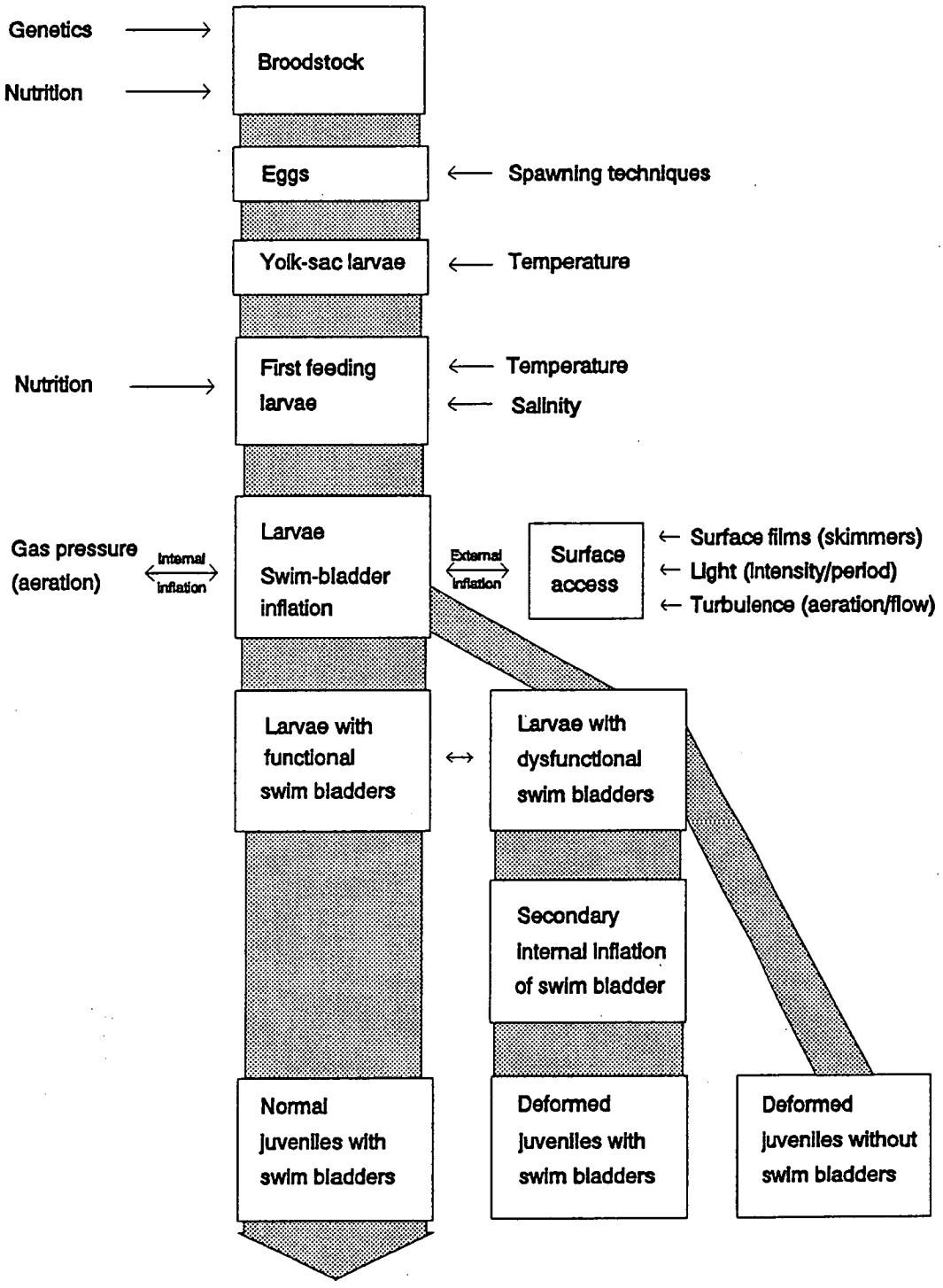

Figure 2 Schematic diagram of factors influencing initial swim bladder inflation in larval marine fish.

early development, growth and survival (Heming and Buddington 1988). In general larger larvae can be expected to be stronger swimmers (Hunter 1972), more resistant to starvation (Blaxter and Hempel 1963), able to start feeding earlier (Wallace and Aasjord 1984), and have increased feeding success (Braum 1967; Ellertsen et al. 1980). Larger larvae also have an increased probability of successful swim bladder inflation as discussed above (Hadley et al. 1987).

Eggs and yolk-sac larvae were incubated in 60 L flow-through incubators similar to those described by Hogan (1988). Larvae were transferred in covered buckets to rearing tanks. Larval rearing trials were conducted intensively in either 10 000 l flat-bottomed tanks or 2000 l conical-bottomed tanks. The latter tanks had biological filters and flow-through salt water exchange. Water quality parameters were measured daily and a sample of 10 larvae were siphoned from the tank daily to monitor feeding, growth and development (Appendix D). Daily assessment of larvae was an important factor in the successful culture of all four species. It was particularly important in the detection of disease as discussed below.

In common with most marine fish hatcheries world-wide, first-feeding larvae were fed rotifers *Brachionus plicatilis* and then brine shrimp *Artemia sp* (Persoone et al. 1980; Fukusho 1989; Sorgeloos et al. 1992). Rotifers were usually fed for the first three weeks and then brine shrimp up until metamorphosis and weaning (Appendix C). Rotifers and brine shrimp were cultured as described by Battaglene and Talbot (1989), Talbot et al. (1990) and Talbot and Battaglene (1991). The density of rotifers fed to first feeding larvae ranged from 5 to 15 rotifers/ml and for brine shrimp from 0.2 to 4.4 nauplii/ml (Appendix C). Similar feeding rates have been used for a wide range of marine fish larvae (see review Tucker 1992a). Van der Wal and Nell (1986) determined that growth and survival of *M. novemaculeata* larvae were best at 9-15 rotifers/ml and 6 brine shrimp/ml. However, it is worth noting that this study may have been conducted on larvae without swim bladders and the live foods were not nutritionally enriched (P. Beevers personal communication, 1989).

Nominal feeding rates are often only of theoretical value (Tucker 1992a) and depend on the density of larvae, tank hydrodynamics and operating procedures. Large strain rotifers were usually fed daily and survival and growth may have been improved if more frequent feeding had been logistically possible. The use of small strain rotifers, not available in Australia during the study, may have improved survival of intensively cultured *S. ciliata* and *P. auratus* because they both have small mouths. In Japan, *Sillago japonica* and *P. auratus* are initially reared on small strain rotifers (Fukusho 1989; Oozeki et al. 1992). In contrast, *M. novemaculeata* were successfully reared on brine shrimp from first feeding to 18-days-old (Appendix C) obviating the expensive rotifer production stage. However, there were more deformities in larvae reared solely on brine shrimp. *Argyrosomus hololepidotus* also has a large mouth and may be capable of consuming brine shrimp at first feed (Chapter 11).

According to Heming and Buddington (1988) most cultured larvae are capable of mixed feeding (exogenous and endogenous), thereby avoiding a potential metabolic deficit before yolk absorption. However the majority of larvae, the exception being *M. novemaculeata,* reared intensively in the current study, started feeding after the completion of yolk-sac absorption and feeding was usually delayed for 24 to 48 h after yolk-sac absorption. Delaying feeding and keeping the larvae in the dark not only assisted swim bladder inflation as discussed above but also conserved rotifer supplies and ensured that nutritionally enriched rotifers were consumed when feeding started. Early feeding can be disadvantageous for other reasons. For example, "precocious" feeding in salmonids gives no advantage in growth or survival and is also considered unprofitable in *S. auratus* because energy expended in search of prey is not compensated for by ingestion due to poor development (Yufera et al.1993). Changes in larval behaviour occur at the end of endogenous feeding as larvae start exogenous feeding (Fukuhara 1987; Skiftesvik 1992). For example, in *G. morhua* and *S. maximus* activity increases whereas swimming speed decreases (Skiftesvik 1992). Keeping larvae in the dark and delaying feeding therefore conserved energy and may also have reduced early size heterogeneity, a particularly important factor for cannibalistic larvae like *A. hololepidotus* and *P. auratus* (Chapters 6 and 11).

Numerous studies have shown the importance of n-3 HUFA in the diet of marine fish larvae (Watanabe et al .1983; Tucker 1992b; Rimmer et al. 1994b). Enriching rotifers and brine shrimp with n-3 HUFA is therefore a requirement for rearing most marine fish larvae (Fukusho 1989; Sorgeloos and Leger 1992). The essential fatty acids for marine fish larvae are generally considered to be the C20 and C22 unsaturated fatty acids, particularly 20:5*n*-3 (EPA) and 22:6*n*-3 (DHA) (Watanabe et al. 1983, 1989; Rimmer et al. 1994b). Some marine fish require both EPA and DHA , although DHA is usually considered more important, it is prudent to provide both in foods for marine fish larvae (Tucker 1992b; Watanabe 1993). Tucker (1992b) recommends a dietary n-3 HUFA content of 2-4%, including at least 1% EPA and 1% DHA in the absence of species-specific information.

The HUFA requirements of *P. auratus* have been the focus of a lot of research in Japan (Kitajima et al. 1980; Watanabe et al. 1983,1989; Foscarini 1988; Izquierdo et al. 1989; Morishita et al. 1989; Tandler et al. 1989b; Watanabe 1993 ; Chapter 6) but much less is known about the requirements of the other three species reared in the current study. Izquierdo et al. (1989) suggested that *P. auratus* larvae require a *n*-3HUFA content of 3.0% in brine shrimp. *Macquaria novemaculeata* have relatively high levels of HUFA in their eggs suggesting they may also require high levels in their diet (Anderson et al. 1990; Battaglene and Anderson unpublished data, 1993).

Reviews of the published n-3 HUFA profiles of rotifers and brine shrimp enriched with a wide variety of algae, yeast and other products are given by Tucker (1992b) and Mourente et al.

(1993). No analysis of diets fed during the current study was undertaken. However, the nutritional profiles of the algal isolates used on the research station are available (Brown et al. 1989; Volkman et al. 1989). Tahitian *Isochrysis* aff. *galbana* and *Pavlova lutheri* were used to enrich live foods on the basis of published studies showing high levels of EPA and DHA (Volkman et al. 1989). The development of techniques for enrichment and the availability of new enrichment products meant that the quality of live feeds increased as the study progressed. This was particularly true for the enrichment of brine shrimp and the results presented in Chapters 6 and 11(Appendix C2{S1} and C4{M1&2}) were obtained using enrichment feeding of brine shrimp with a microencapsulated diet high in polyunsaturated fatty acids (Frippak 'car#1' Chapter 5). Subsequent trials used a superior brine shrimp boosting product (high DHA Super Selco, Artemia Systems NV Belgium), combined with a specially formulated marine fish larval weaning diet (ML diets, Fukui, Yokohama, Japan). In addition, small nutritionally superior brine shrimp of guaranteed quality (AF brand, Artemia Systems) were used for the first few days of brine shrimp feeding in latter trials. The larval mortality of all species, but particularly *P. auratus* at weaning (Chapter 6), appeared greatly reduced using these improved methods (Battaglene et al. 1993).

The improvement in larval survival was difficult to quantify in production trials because of the confounding influences of different initial stocking densities, cannibalism and disease outbreaks. Initial stocking densities ranged from very low <1 larvae/l to as high as 100/l (Table 4). Typical initial stocking densities for production of intensively cultured larvae of the same or similar species range from 12 to 72 larvae/l for *P. auratus* (Fukusho 1989), 10-20/l for *Sciaenops ocellatus* (Holt et al. 1990), 30/l for *S. japonica* (Oozeki et al. 1992), 100/l for *D. labrax* and *S. auratus* (Chatain and Ounais-Guschemann 1990) and 1000/l for *M.* saxatilis (Nicholson et al. 1990). A reduction in larval density is usually required at or before weaning depending on the initial number of larvae stocked, the survival rate and larval behaviour. Cannibalism of larvae was particularly evident for *P. auratus* and *A. hololepidotus* at weaning (Battaglene 1994; Chapters 6 and 11).

Cannibalism is regarded as an alternative feeding strategy, more likely to be adopted by larvae and early juveniles which are carnivorous, when resources become limiting (Hecht and Pienaar 1993). It is a major problem in the culture of many marine fish larvae. Size variation is a primary cause and an effect of cannibalism and agonistic behaviour in larval fish (MacKinnon 1985; Katavic et al. 1989; Hecht and Pienaar 1993). Other factors influencing cannibalism include food and larval density, feeding frequency, light intensity, water clarity, and shelter (see review by Hecht and Pienaar 1993). Cannibalism was controlled in the current study by reducing larval densities, increasing feeding frequencies (Appendix C), removing dominant individuals, regular grading and keeping the larvae in the dark when food was unavailable or in short supply. The use of dark covers at night and before feeding in the morning increased growth and survival of *P. auratus* and *A. hololepidotus,* presumably by

reducing cannibalism and agonistic behaviour. Intensively reared *S. ciliata* were not cannibalistic (Chapter 10) and consequently easier to wean.

Weaning, the transfer from live food to artificial foods is successful with most marine fish with a completely developed digestive tract (Person Le Ruyet et al. 1993). In the current study weaning was undertaken after metamorphosis, initially using chopped fish and squid (Chapters 6 and 11) and in latter trials with commercial microparticles (Appendix C). The weaning strategy used varied slightly between trials but always involved a gradual transfer over a minimum of five days (Appendix C). Similar weaning strategies are used with *P. auratus* in Japan (Foscarini 1988; Kanazawa et al. 1989), although abrupt replacement is favoured with *D. labrax* in Europe (Person Le Ruyet 1990). *Sillago ciliata* proved the easiest fish to wean, followed by *A. hololepidotus* and *P. auratus*. Earlier research had indicated that it was very difficult to wean *M. novemaculeata* (S. Battaglene unpublished data, 1989) and weaning was not carried out with this species during the current study, principally because fish were being produced in ponds for release back into the wild.

Initial low density larval rearing trials were not affected by disease. However, bacterial outbreaks occurred in high density trials with *P. auratus* and *A. hololepidotus*, particularly in 1994 (Appendix C). The bacteria were identified as belonging to the *Vibrio* group, and later tentatively identified as *V. tubiashii* (S . Nearhos unpublished data, 1993). *Vibriosis* is the most significant infectious disease occurring in marine fishes regardless of age and causes losses of 219.5 tonnes p.a. in *P. auratus* in Japan (Hirano and Yone 1972; Sano and Fukuda 1987). Rapid diagnosis and selection of an appropriate antibiotic, usually oxolinic acid (bath of 5mg/l for 5 days) proved an effective control mechanism against bacterial disease in experimental trials. The first larval run of a season was usually free of disease and in 1994 successive trials became infected at an earlier stage suggesting contaminated biofilters may have contributed to the outbreaks. Longer term solutions to the problem of disease control may include increasing hygiene and water quality through the use of UV sterilisers, improved rearing methods, less reliance on biofiltration and the use of vaccines.

4. IMPLICATIONS FOR PROVISION OF SEED STOCK

The research reported in this thesis demonstrates that four native Australian temperate species can be bred in at least moderately large numbers using hormone-induced ovulation of wild-caught broodstock and intensive larval rearing techniques. The thesis identifies a number of 'bottlenecks' in the provision of seed stock. These bottlenecks are different for each species and reflect the relative difficulty of culturing each species and the varying amount of research conducted on each. Development of breeding techniques are most advanced for *M. novemaculeata*, followed by, *S. ciliata, P.auratus* and *A. hololepidotus*.

There is currently no major impediment in the provision of *M. novemaculeata* juveniles. The

commercial production of *M. novemaculeata* for release into farm dams and impoundments is currently undertaken using the methods described in Chapters 2 to 5. However, the commercial farming of *M. novemaculeata* has not been undertaken, partly because of reportedly slow growth rates and difficulties in weaning *M. novemaculeata* on to artificial diets.

The main problem in the provision of *S. ciliata* seed stock is in early larval rearing. High mortality of first-feeding larvae was identified in Chapter 10 and requires further research. The results presented suggest that the use of small strain rotifers may increase survival. However, the techniques described in Chapters 9 and 10 will allow sufficient production of juveniles for the farming potential of *S. ciliata* to be assessed on a pilot scale .

With *P. auratus* the main bottleneck in production is in the hormone-induced ovulation of wild broodstock. Poor egg supplies have limited seed stock production in commercial hatcheries. The techniques described in Chapters 6 to 8 address this problem. The results suggest that the long-term solution to the reliable supply of *P. auratus* eggs is the controlled spawning of domesticated stocks. Improvements in larval rearing techniques described in Chapters 6 and 7 have resulted in marked improvements in survival of intensively cultured *P. auratus* larvae (Appendix C).

The reliable supply of mature broodstock was a limitation in determining the potential of *A. hololepidotus*. However, from the small number of fish treated, it was relatively easy to induce ovulation in both wild and captive broodstock . Similarly, the intensive larval rearing of *A. hololepidotus* was relatively straightforward, although problems with cannibalism did occur in the latter stages of rearing.

The problems experienced with the supply of fertilised eggs from wild-caught *P. auratus* and *A. hololepidotus* broodstock are currently the focus of a large research program undertaken by NSW Fisheries in association with the Department of Aquaculture, University of Tasmania with support from the Cooperative Research Centre for Aquaculture. This research program was formulated in response to the problems identified in this thesis. Another area of research, highlighted in the thesis (Chapters 5 and 11), which should be pursed is the possible advantages of extensive culture. The successful combination of intensive and extensive culture described for *M. novemaculeata* in Chapter 5 and currently used to produce *Lates calcarifer* in northern Australia (Rutledge and Rimmer 1991) could be extended to *A. hololepidotus* and *S. ciliata,* and possiby *P. auratus*.

The techniques described in this thesis have been used to produce over 100 000 *M. novemaculeata*, 30 000 *P. auratus*, 15 000 *A. hololepidotus* and 10 000 *S. ciliata.* Both *P.auratus* and *A. hololepidotus* juveniles have been stocked in sea cages and grown to market size within two years (O' Sullivan 1994).

Addendum

Kraul, S., 1983. Results and hypotheses for the propagation of the grey mullet, *Mugil cephalus* L. Aquaculture, 30:273-284.

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APPENDIX A1 A summary of hormone induction trials with snapper *Pagrus auratus* at the Port Stephens Research Centre 1990 to 1994

Ref no.	Year	Month	Weigh (g)	TL (mm)	Sex	Stage	Hormone Treatment	Oocyte (um)	Ovulation	Batch	Latent period (h)	No. of eggs	Fert (%)
1	1990	10	1108	335	F	V	HCG1000	319	NO				
2	1990	10	1260	385	F	V	HCG1000	452	NO				
3	1990	10	914	342	M	VI	HCG200						
4	1990	10	850	337	F	II	HCG1000	181	NO				
5	1990	10	1341	374	F	V	HCG1000	560	NO				
6	1990	10	1104	370	M	VI	HCG200						
7	1990	10	1404	395	F	V	HCG1000	554	NO				
8	1990	10	1389	400	M	VI	HCG200						
9	1990	11	3200	516	F	VI	HCG1000	1000	NO				
10	1990	11	3900	550	M	VI	HCG200						
11	1990	11	1066	373	F	II	NIL	50	NO				
12	1990	11	878	340	M	V	NIL						
13	1990	11	1517	400	F	VI	HCG1000	1058	YES	1	24.5		
14	1990	11	964	350	M	VI	HCG200						
15	1990	11	2258	468	F	VI	HCG1000	1135	YES	1	24.5		26
16	1990	11	1206	379	M	VI	HCG200						
17	1990	11	827	340	F	II	NIL		NO				
18	1990	11	946	340	F	II	NIL		NO				
19	1990	11	948	342	F	V	HCG1000	892	YES	1	24	4000	0
20	1990	11	2000	464	M	VI	HCG1000						
21	1990	11	2000	479	F	V	HCG1000	1028	YES	1	24.5	1000	0
22	1991	9	925	350	M	IV	NIL						
23	1991	9	870	346	M	IV	NIL						
24	1991	9	1490	412	M	VI	NIL						
25	1991	9	1250	387	F	V	HCG1000	1000	YES	1	24	6500	4
26	1991	9	1450	409	F	V	HCG1000	1200	YES	2	24	15000	33 to 90
27	1991	10	1200	376	M	V	HCG200						
28	1991	10	1250	390	M	V	HCG200						
29	1991	10	2250	466	F	V	HCG1000	1100	YES	3	TO	206475	3 to 10
30	1991	10	1740	420	F	V	HCG1000	650	YES	2	TO	4800	0 to 76
31	1991	10	1150	372	M		NIL						
32	1991	10	2050	462	F	V	OVA	990	YES	5	TO	229375	0 to 30
33	1991	10	1000	343	M	VI	NIL						
34	1991	10	1040	366	F	V	OVA	760	YES	7	TO	91771	0 to 73
35	1991	10	1880	393	M	V	HCG200						
36	1991	10	2200	473	F	V	HCG1000	1120	YES	1	24	40000	0
37	1991	10	2240	480	M	VI	NIL						
38	1991	10	1220	394	M	V	HCG200						
39	1991	10	1050	359	M	VI	NIL						
40	1991	10	810	330	M	V	HCG200						
41	1991	10	1300	398	M	V	HCG200						
42	1991	10	810	324	M	V	HCG200						
43	1991	10	750	323	M		NIL						
44	1991	10	2550	488	F		NIL						
45	1991	10	3240	545	M		NIL						
46	1991	11	940	342	?	I	NIL						
47	1991	11	1580	422	F	V	OVA	900	NO				
48	1991	11	1100	358	M	VI	HCG1000						
49	1991	11	840	333	F	II	NIL						

50	1991	11	2050	473	M	VI	HCG1000						
51	1991	11	3600	580	M		NIL						
52	1991	11	2300	486	M		NIL						
53	1991	11	1100		M		NIL						
54	1991	11	850	330	M		NIL						
55	1991	11	2000	463	F	VI	HCG1000						
56	1991	11	2300	480	F	VI	NIL	1100	YES	1	0	38709	?
57	1991	11	700		M	II	NIL						
58	1991	11	1060	363	?	I	NIL						
59	1991	11	1360	413	M	VI	OVA						
60	1991	11	1100	370	?	I	NIL						
61	1991	11	1000		M		NIL						
62	1991	11	1650	425	M		OVA						
63	1991	11	1230	393	M		NIL						
64	1991	11	1420	399	M		OVA						
65	1991	11	3050	540	F		OVA	900	YES	3	TO	159380	0 to 25
66	1991	11	1080	374	M		OVA						
67	1992	10	600	300	F	II	NIL						
68	1992	10	500	280	F	II	NIL						
69	1992	10	500	281	F	II	NIL						
70	1992	10	400	257	M	II	NIL						
71	1992	10	2480	497	M	VI	HCG200						
72	1992	10	3200	536	F	V	HCG1000	549	YES	7	TO	558656	7 to 98
73	1992	11	4600	665	M	VI	NIL						
74	1992	11	1600	415	F	V	HCG1000	1150	YES	1	18	44000	0
75	1992	11	1900	452	F	V	HCG1000	1170	YES	4	TO	176165	0 to 96
76	1992	11	1150	365	M	VI	HCG200						
77	1992	11	1140	363	F	II	NIL						
78	1992	11	750	330	F	II	NIL						
79	1992	11	1000	354	F	II	NIL						
80	1992	11	700	322	M	II	NIL						
81	1992	12	4500	615	M	VI	NIL						
82	1992	12	4900	655	M	V	NIL						
83	1992	12	2200	454	M	II	NIL						
84	1992	12	2200	470	M	VI	NIL						
85	1992	12	3000	523	F	V	HCG500	890	YES	1	20	5000	0
86	1992	12	450	340	M	II	NIL						
87	1992	12	1450	390	M	IV	NIL						
88	1992	12	800	320	F	II	NIL						
89	1992	12	900	332	M	II	NIL						
90	1993	8	650	324	M	VI	HCG1000						
91	1993	8	650	323	F	II	NIL						
92	1993	8	650	320	F	V	HCG1000	511	NO				
93	1993	8	700	339	M	II	NIL						
94	1993	8	700	351	F	VI	HCG1000	978	YES	4	TO	17475	0 to 87
95	1993	8	700	335	M	VI	HCG1000						
96	1993	8	600	304	F	III	NIL						
97	1993	8	600	314	M	VI	HCG1000						
98	1993	8	750	336	F	V	HCG1000	477	NO				
99	1993	8	700	330	M	VI	HCG1000						
100	1993	8	1150	399	M	VI	HCG250						
101	1993	8	2400	474	F	IV	HCG1000	643	NO				
102	1993	8	1200	372	F	IV	HCG1000	554	YES	5	TO	104616	9 to 69
103	1993	8	650	306	M	VI	HCG1000						
104	1993	9	700	308	F		HCG1000	900	YES	5	TO	10000	0 to 100

160	1993	11	1700	440	M	VI	HCG250						
161	1993	11	3400	533	M	VI	HCG250						
162	1993	11	850	370	M	VI	HCG500						
163	1993	11	800	384	M		NIL						
165	1993	11	1000	378	M		NIL						
166	1993	11	1400	394	F	II	NIL						
167	1993	11	1200	382	F	V	HCG500	945	YES	5	TO	77046	0 to 18
168	1993	11	700	332	F	II	NIL						
169	1993	11	2300	492	F	V	HCG1000	1120	YES	1	8	7590	0
170	1993	11	5400	656	F	V	HCG500	1117	NO				
171	1993	11	700	347	M	V	NIL						
172	1993	11	2100	467	M	VI	HCG500						
173	1993	11	700	311	M	VI	HCG500						
174	1993	11	800	319	F	II	NIL						
175	1993	11	650	298	M	III	NIL						
176	1993	11	2000	460	F	V	HCG500	926	NO				
177	1993	11	1800	445	M	VI	HCG500						
178	1993	11	2700	497	F	V	HCG500	920	NO				
179	1993	12	700	336	M	VI	OVA						
180	1993	12	650	325	F	V	HCG1000	1000	YES	3	TO	56372	0 to 31
181	1993	12	700	344	M	VI	HCG1000						

105	1993	9	670	301	M	III	NIL						
106	1993	9	650	300	F	II	NIL						
107	1993	9	1500		F		HCG1000	972	NO				
108	1993	9	750	326	M		HCG1000						
109	1993	9	1000	348	M		HCG1000						
110	1993	9	2560	480	M		NIL						
111	1993	9	760	345	M		NIL						
112	1993	9	3400	558	M		NIL						
113	1993	9	2200	473	F	VI	HCG1000		YES	1	0	16032	94
114	1993	9	750	363	M		NIL						
115	1993	9	2050	458	F		HCG1000	578	YES	3	TO	6000	18 to 76
116	1993	9	610	314	F	II	NIL						
117	1993	9	740	301	M	II	NIL						
118	1993	9	630	288	F	II	NIL						
119	1993	11	650	334	M	II	NIL						
120	1993	11	3100	542	M	VI	NIL						
121	1993	11	1650	419	M	VI	NIL						
122	1993	11	750	369	F	V	HCG1000	978	YES	3	TO	3200	0 to 88
123	1993	11	750	345	F	II	NIL						
124	1993	11	650	318	F	II	NIL						
125	1993	11	700	322	M	VI	NIL						
126	1993	11	1100	363	F	II	NIL						
127	1993	11	1700	420	M		NIL						
128	1993	11	650	357	M		NIL						
129	1993	11	700	368	M	III	NIL						
130	1993	11	700	325	F	II	NIL						
131	1993	11	800	354	M	VI	NIL						
132	1993	11	1900	460	F	II	NIL						
133	1993	11	1000	368	M	I	NIL						
134	1993	11	2000	462	F	V	HCG2500	538	NO				
135	1993	11	1300	390	F	II	NIL						
136	1993	11	1150	390	M	VI	HCG1000						
137	1993	11	1150	410	F		NIL						
138	1993	11	1100	382	F	II	NIL						
139	1993	11	700	328	F	III	NIL						
140	1993	11	700	348	F	V	HCG1000	969	YES	3	TO	19200	0 to 31
141	1993	11	1700	437	M	VI	HCG1000						
142	1993	11	800	365	F	II	NIL						
143	1993	11	700	343	F	II	NIL						
144	1993	11	500	288	?		NIL						
145	1993	11	650	315	F	II	NIL						
146	1993	11	800	368	F	V	HCG1000	1090	YES	2	TO	84800	0 to 6
147	1993	11	750	378	M	II	NIL						
148	1993	11	2200		F	VI	HCG500	1200	YES	4	TO	129506	0 to 86
149	1993	11	850	346	M	VI	HCG200						
150	1993	11	700	347	?		NIL						
151	1993	11	700	332	M	II	NIL						
152	1993	11	650	322	F	II	NIL						
153	1993	11	700	353	F	VI	HCG500	UN	YES	4	TO	47227	0 to 91
154	1993	11	700	346	F	II	NIL						
155	1993	11	700	318	F	II	NIL						
156	1993	11	950	341	M	III	NIL						
157	1993	11	800	332	M	III	NIL						
158	1993	11	700	332	M	VI	NIL						
159	1993	11	1200	380	M	VI	HCG1000						

Summary hormone induction trials with snapper *Pagrus auratus*
From Fisheries Research Station pool

Ref no.	Year	Month	Weight (g)	TL (mm)	Sex	Stage	Hormone Treatment	Oocyte (um)	Ovulation	Batch	Latent period	No. of eggs	Fert (%)
1	1990	10	2637	485	F	IV	HCG200	97	NO				
2	1990	10	2904	530	F	IV	HCG1000	334	NO				
3	1990	10	2355	489	M	V	HCG200						
4	1990	10	1934	450	F	II	NIL	46					
5	1990	10	2424	510	M	VI	HCG200						
6	1990	10	2561	479	F	II	NIL	62					
7	1990	10	2010	454	M	VI	NIL						
8	1991	1	3150	512	F								
9	1991	1	2600	500									
10	1991	1	3500	544	M	VI							
11	1991	1	3150	513	M	VI							
12	1991	1	2550	480	M	VI							
13	1991	1	2250	475	M	VI							
14	1991	1	2850	492	M	VI							
15	1991	1	2450	475									
16	1991	1	2325	478									
17	1991	1	3300	538	F								
18	1991	1	3275	527									
19	1991	1	3000	522									
20	1991	1	2750	500									
21	1991	1	3050	500									
22	1991	1	3000	505									
23	1991	1	1650	412									
24	1991	1	2350	453	F								
25	1991	1	2575	500									
26	1991	1	2800	489									
27	1991	1	2250	455									
28	1991	1	3250	528									
29	1991	1	2825	523									
30	1991	1	1850	428	F								
31	1991	1	2350	475	M	VI							
32	1991	1	2425	483	F								
33	1991	1	2275	458									
34	1991	1	3150	507									
35	1991	1	2450	480									
36	1991	1	2300	482									
37	1991	7	1350	380	F	IV	NIL	496	NO				
38	1991	7	800	323	M	VI	NIL						
39	1991	7	1150	370	F	IV	NIL	446	NO				
40	1991	7	1050	357	M	VI	NIL						
41	1991	7	950	338	F	IV	NIL	492	NO				
42	1991	7	1000	346	M	VI	NIL						
43	1991	9	580	304	F	II	NIL	269	NO				
44	1991	9	1070	366	F	IV	NIL	457	NO				
45	1991	9	1100	364	M	VI	NIL						
46	1991	9	950	347	F	II	NIL						

47	1991	9	1000	340	F	IV	NIL	495	NO				
48	1991	10	1240	374			NIL						
49	1991	10	1040	360			NIL						
50	1991	10	1270	380	M	VI	HCG250						
51	1991	10	1040	351	F	II	NIL	150					
52	1991	10	1290	382	M	III	NIL						
53	1991	10	1220	377	M	III	NIL						
54	1991	10	1100	361			NIL						
55	1991	10	1380	368	M	VI	NIL						
56	1991	10	990	350	M	III	NIL						
57	1991	10	980	349	M	III	NIL						
58	1991	10	1290	380	M	VI	HCG200						
59	1991	10	1150	358	M	III	OVAPRIM						
60	1991	10	850	334	M	III	NIL						
61	1991	10	800	323	M	VI	OVAPRIM						
62	1991	10	1150	369	M	III	NIL						
63	1991	10	1100	358			NIL						
64	1991	10	1000	342	M	VI	NIL						
65	1991	10	1160	370	M	III	HCG200						
66	1991	10	1250	380			NIL						
67	1991	10	1400	384	F	II	OVAPRI	135	NO				
68	1991	10	850	328	M	III							
69	1991	10	680	300	F	II							
70	1991	10	900	326	F			275					
71	1991	10	900	335	F			158					
72	1991	10	740	322	F			197					
73	1991	10	1160	370	M	III							
74	1991	10	1050	345	F		OVAPRI	457					
75	1991	10	1000	337	F			158					
76	1991	10	1040	345	M	VI	OVAPRIM						
77	1991	10	1160	368	M	VI	OVAPRIM						
78	1991	10	650	290	M	VI	OVAPRIM						
79	1991	10	1080	354	M	VI							
80	1991	10	860	329	M	VI							
81	1991	10	800	333	M	VI							
82	1991	10	1070	355	F			158					
83	1991	10	1025	345	F			158					
84	1991	10	980	345	F			118					
85	1991	10	1240	379	M	111							
86	1991	10	1520	400	M	VI							
87	1991	10	1020	345	M	VI							
88	1991	10	1370	375	M	VI							
89	1991	10	900	330	M	VI							
90	1991	10	890	330	M	VI							
91	1991	10	960	344	M	VI							
92	1991	10	1200	368	M	VI							
93	1991	11	3100	558			NIL						
94	1991	11	3450	542	F	II	NIL						
95	1991	11	2460	490	F	II	NIL						
96	1991	11	2500	500			NIL						
97	1991	11	2710	505			NIL						

98	1991	11	3450	570	F	II	NIL						
99	1991	11	3700	575	M		NIL						
100	1991	11	2900	519	F	III	NIL	368					
101	1991	11	2280	474	M		NIL						
102	1991	11	3340	529	M	VI	NIL						
103	1991	11	2900	510	M	VI	NIL						
104	1991	11	2620	513	M	VI	NIL						
105	1991	11	3230	536	M	VI	NIL						
106	1991	11	2100	449	M	VI	NIL						
107	1991	11	3120	525	M	VI	NIL						
108	1991	11	2910	528	M	VI	NIL						
109	1991	11	2150	357	M	VI	NIL						
110	1991	11	920	345	M	VI	NIL						
111	1991	11	1350	385	M	VI	NIL						
112	1991	11	1050	347	M		NIL						
113	1991	11	1100	363	F	II	NIL						
114	1991	11	1200	360	F	II	NIL						
115	1991	11	1000	344			NIL						
116	1991	11	1280	365	M	VI	NIL						
117	1991	11	920	332	M	IV	NIL						
118	1991	11	950	340	M	VI	NIL						
119	1991	11	920	330			NIL						
120	1991	12	1620	405	M								
121	1991	12	1310	377	M								
122	1991	12	1240	366									
123	1991	12	1350	389									
124	1991	12	1310	384									
125	1991	12	1590	407	M								
126	1991	12	1200	376									
127	1991	12	1050	350	F								
128	1991	12	1440	395									
129	1991	12	1320	388	M								
130	1991	12	1380	394	M								
131	1991	12	1460	405									
132	1991	12	1540	401									
133	1991	12	1400	395									
134	1991	12	1300	387									
135	1991	12	1607	413	M								
136	1991	12	1160	366									
137	1991	12	1400	390	M								
138	1991	12	1150	357									
139	1991	12	1380	390	M								
140	1991	12	1600	405									
141	1991	12	1120	363									
142	1991	12	1110	367									
143	1991	12	1260	375									
144	1991	12	1760	434									
145	1991	12	1000	350	M								
146	1992	9		385	F	III	NIL	397	NO				
147	1992	9		433	F	III	NIL	430	NO				
148	1992	9	1600	424	F	II	NIL	276	NO				

149	1992	9	1150	380	F	II	NIL	236	NO				
150	1992	9	1550	408	F	II	NIL	237	NO				
151	1992	9	1250	394	F	II	NIL	278	NO				
152	1992	9	1240	379	F	I	NIL	40	NO				
153	1992	9	1900	448	F	I	NIL	UN	NO				
154	1992	9	1300	400	F	I	NIL	UN	NO				
155	1992	9	1200	390	F	III	NIL	316	NO				
156	1992	11	2300	460	F	II							
157	1992	11	2000	448									
158	1992	11	2550	490	F	II							
159	1992	11	2400	470									
160	1992	11	1600	410									
161	1992	11	2300	453									
162	1992	11	1600	405									
163	1992	11	2200	454									
164	1992	11	2200	442									
165	1992	11	1950	452									
166	1992	11	2400	480									
167	1992	11	1950	438									
168	1992	11	1700	408									
169	1992	11	1800	428									
170	1993	9	2455	457	F	V	HCG1000	590	NO				
171	1993	9	1610	418	F	V	HCG1000	601	YES	1	92	22770	83
172	1993	9	2064	479	F	V	HCG1000	570	YES	2	9 TO 9	78430	1 TO 33
173	1993	9	1800	430	F	V	HCG1000	642	NO				
174	1993	9	1684	424	F	V	HCG1000	604	YES	4	9 TO 8	50600	2 TO 22
175	1993	9	1262	376	F	V	HCG1000	562	NO				
176	1993	10	1550	423	M	VI							
177	1993	10	1740	427	M	VI							
178	1993	10	1920	460	M	VI							
179	1993	10	1500	428	M	VI							
180	1993	10	2000	459	M	VI							
181	1993	10	1800	445	M	VI							
182	1993	10	1600	426	M	VI							
183	1993	10	1480	413	M	VI							
184	1993	10	1160	436	?								
185	1993	10	1920	459	M	VI							
186	1993	10	2160	490	?								
187	1993	10	1700	438	M	VI							
188	1993	10	1600	426	?								
189	1993	10	1900	454	F								
190	1993	10	2280	470	M	VI							
191	1993	10	1900	430	M	VI							
192	1993	10	1600	432	M	VI							
193	1993	10	1109	349	M	VI							
194	1993	10	1400	407	M	VI							
195	1993	10	1400	407	M	VI							
196	1993	10	2030	464	F	III		308					
197	1993	10	1380	410	?								
198	1993	10	1920	448	F	V	HCG1000	500	NO				
199	1993	10	2300	494	M	VI							

200	1993	10	1620	412	F	III		308					
201	1993	10	1900	450	M	VI							
202	1993	10	1660	430	M	VI							
203	1993	10	1750	440	M	VI							
204	1993	10	2200	480	F	II		154					
205	1993	10	1550	373	F	II		154					
206	1993	10	1400	408	M	VI							
207	1993	10	1710	443	M	VI							
208	1993	10	1700	431	M	VI							
209	1993	10	2050	455	M	VI							
210	1993	10	1900	408	M	VI							
211	1993	10	1600	435	F	II		239					
212	1993	10	1450	410	F	V	HCG1000	547	NO				
213	1993	10	1500	420	F	I		100					
214	1993	10	2600	489	M	IV							
213	1993	10	1600	421	M	VI							
214	1993	10	1440	387	F	V	HCG1000	481	NO				
215	1993	10	1810	468	?								
216	1993	10	1380	430	F	V	HCG1000	485	NO				
217	1993	10	1060	391	F	V	HCG1000	411	NO				
218	1993	10	1320	433	F	I		100					
219	1993	10	2000	457	F	I		100					
220	1993	10	1450	410	M	VI							
221	1993	10	2000	462	F	I		100					
222	1993	10	1150	390	M	VI							
223	1993	10	2000	460	M	VI							
224	1993	10	1710	431	F	III		385					
225	1993	10	1600	413	F	III		385					
226	1993	10	1500	418	F	I		100					
227	1993	10	1850	448	M	VI							
228	1993	10	1700	415	M	VI							
229	1993	10	2200	455	M	VI							
230	1993	10	1720	434	F	II		231					
231	1993	10	1820	448	F	V	HCG1000	477	NO				
232	1993	10	1160	378	M	VI							
233	1993	10	2550	510	F	I		100					
234	1993	10	1450	410	F	V	LHRHa	450	YES	8	7 TO 2	153696	0 TO 56
235	1993	10	1550	430	F	V	LHRHa	454	YES	1	211	5940	13
236	1993	10	1810	430	F	V	LHRHa	408	YES	5	0 TO 2	199986	1 TO 75
237	1993	10	2000	470	F	V	LHRHa	516	YES	1	163	253506	0
238	1993	10	1700	420	F	I		100					
239	1993	10	1400	398	F	V	LHRHa	493	YES	1	163	147752	0
240	1993	10	1860	428	M	VI							
241	1993	10	2500	488	F	I		100					
242	1993	10	1450	407	M	VI							
243	1993	10	2000	460	F	V	OVAPRI	447	NO				
244	1993	10	1550	398	F	I		100					
245	1993	10	1770	440	M	VI							
246	1993	10	2700	517	M	VI							
247	1993	10	2050	462	F	II							
248	1993	10	1800	446	M	VI							

249	1993	10	1760	437	M	VI							
250	1993	10	1820	435	F	V	OVAPRI	543	NO				
251	1993	10	1720	430	F	II							
252	1993	10	1650	440	M	?							
253	1993	10	1650	438	M	VI							
254	1993	10	1900	445	M	VI							
255	1993	10	2300	488	M	VI							
256	1993	10	1850	450	M	VI							
257	1993	10	1750	432	M	VI							
258	1993	10	2200	470	F	I		100					
259	1993	10	1820	440	F	II							
260	1993	10	1600	423	F	II							
261	1993	10	1650	437	F	II							
262	1993	10	1650	422	M	VI							
263	1993	10	1500	430	M	VI							
264	1993	10	2200	462	M	IV							
265	1993	10	1570	410	M	IV							
266	1993	10	1550	414	F	V	OVAPRI	450	NO				
267	1993	10	1500	410	M	VI							
268	1993	10	1750	432	M	VI							
269	1993	10	1680	419	M	VI							
270	1993	10	1970	461	F	II							
271	1993	10	2150	468	F	II							
272	1993	10	2000	460	?	II							
273	1993	10	1650	412	F	II							
274	1993	10	1300	385	F	V	OVAPRI	466	NO				
275	1993	10	1650	423	F	V	OVAPRI	616	YES	3	0 TO 6	1 TO 16	NA
276	1993	10	1700	435	?								
277	1993	10	2000	458	F	II							
278	1993	10	2100	468	?								
279	1993	10	2000	448	M	VI	LHRH						
280	1993	10	2000	466	F	II							
281	1993	10	1700	427	F	II							
282	1993	10	1700	426	M	VI	LHRH						
283	1993	10	2100	472	M	VI	LHRH						
284	1993	10	1500	434	M	VI	LHRH						
285	1993	10	1800	442	?								
286	1993	10	2200	474	M	VI	LHRH						
287	1993	10	1000	385	F	II							
288	1993	10	2100	479	M	VI	LHRH						
289	1993	10	1800	460	?								
290	1993	10	2000	464	?								
291	1993	10	2200	480	M	VI							
292	1993	10	2000	465	?								
293	1993	10	1500	423	M	VI							
294	1993	10	1300	398	?								
295	1993	10	1500	410	F	II							
296	1993	10	2000	457	M	VI							
297	1993	10	1600	427	F	II							
298	1993	10	1500	423	?								
299	1993	10	2400	485	F	II							

300	1993	10	2400	540	?								
301	1993	10	2000	460	F	II							
302	1993	10	1800	438	F	II							
303	1993	10	1500	425	?								
304	1993	10	4500	595	F	II							
305	1993	10	4500	600	M	VI							
306	1993	10	4000	575	M	VI							
307	1993	10	4300	614	M	VI							
308	1993	10	4000	582	F	II							
309	1993	10	1700	408	F	II							
310	1993	10	3100	530	F	II							
311	1993	10	3900	557	F	II							
312	1993	10	4300	600	F	II							
313	1993	10	2500	488	M	V							
314	1993	10	4900	631	M	VI							
315	1993	10	4500	620	M	VI							
316	1993	10	3600	548	M	VI							
317	1993	10	4700	646	M	VI							
318	1993	10	4850	625	F	II							

APPENDIX A2 A summary of hormone induction trials with sand whiting *Sillago ciliata* at the Port Stephens Research Centre 1991to 1993

Ref no.	Year	Month	Weigh (g)	TL (mm)	Sex	Stage	Hormone treatment	Oocyte (um)	vulatio	Batch	Latent period (h)	No. of eggs	Fert (%)
1	1991	12	200	263	F	V	OVAPRIM		YES	1	49	21120	0
2	1991	12	150	244	F	II	OVAPRIM		NO				
3	1991	12	200	266	F	II	OVAPRIM		NO				
4	1991	12	200	260	F	II	HCG300		NO				
5	1991	12	160	237	M	VI	HCG300						
6	1992	1	200	262	M	VI	NIL						
7	1992	1	160	248	F		HCG300	340	NO	0			
8	1992	1	140	229	M	VI	HCG350						
9	1992	1	200	250	F		HCG300	430	YES	1	40.5	25300	25
10	1992	1	170	254	M	VI	HCG300						
11	1992	1	130	222	M	VI	NIL						
12	1992	1	150	234	F		OVA	190	NO				
13	1992	1	160	235	M	VI	OVA						
14	1992	2	171	262	M	VI	OVA						
15	1992	2	160	250	M	VI	NIL						
16	1992	2	235	290	M	VI	HCG300						
17	1992	2	201	273	M	VI	HCG300						
18	1992	2	227	280	M	VI	OVA						
19	1992	2	165	257	M	VI	HCG300						
20	1992	2	155	247	M	VI	HCG300						
21	1992	2	132	233	M	VI	NIL						
22	1992	2	256	300	M	VI	NIL						
23	1992	2	152	248	M	VI	OVA						
24	1992	2	218	283	M	VI	OVA						
25	1992	2	140	243	M	VI	NIL						
26	1992	2	134	240	M	VI	HCG300						
27	1992	2	165	247	M	VI	OVA						
28	1992	2	197	267	M	VI	HCG300						
29	1992	2	142	251	M	VI	HCG300						
30	1992	2	181	266	M	VI	OVA						
31	1992	2	200	272	M	VI	OVA						
32	1992	2	139	245	M	VI	HCG300						
33	1992	2	186	258	M	VI	OVA						
34	1992	2	300	296	F	V	OVA	430	NO				
35	1992	2	297	305	F	V	NIL	420	NO				
36	1992	2	355	317	F	V	HCG300	430	YES	1	36.8	266962	92
37	1992	2	287	305	F	V	HCG300	430	YES	1	36.7	66118	96
38	1992	2	252	300	F	V	OVA	440	YES	1	47.8	4640	33
39	1992	2	261	307	F	V	HCG300	730	NO				
40	1992	2	299	310	F	V	HCG300	450	NO				
41	1992	2	266	293	F	V	NIL	440	NO				
42	1992	2	200	267	F	V	NIL	420	NO				
43	1992	2	215	275	F	V	OVA	420	YES	1	31.7	39987	97
44	1992	2	274	300	F	V	OVA	440	YES	2	31.7	55993	98
45	1992	2	230	279	F	V	NIL	460	NO				
46	1992	2	248	290	F	V	HCG300	450	YES	1	34.5	104486	68
47	1992	2	248	295	F	V	OVA	440	YES	1	32.2	17005	97
48	1992	2	229	280	F	V	HCG300	430	NO				

49	1992	2	205	280	F	V	HCG300	430	NO				
50	1992	2	229	287	F	V	OVA	440	YES	2	35.8	22399	92
51	1992	2	175	258	F	V	OVA	420	NO				
52	1992	2	218	280	F	V	HCG300	440	NO				
53	1992	2	260	295	F	V	OVA	420	YES	2	32.1	28324	59
54	1993	1	115	227	M	VI	HCG300						
55	1993	1	151	248	M	VI	HCG300						
56	1993	1	187	268	M	VI	HCG300						
57	1993	1	149	240	M	VI	HCG300						
58	1993	1	173	252	M	VI	HCG300						
59	1993	1	226	277	M	VI	HCG300						
60	1993	1	156	252	M	VI	HCG300						
61	1993	1	170	250	M	VI	HCG300						
62	1993	1	550	375	F	VI	RR		YES	1	0	62570	85
63	1993	1	252	297	M	VI	NIL						
64	1993	1	400	326	F		HCG300	430	YES	2	,35.	203354	76,30
65	1993	1	350	319	F	VI	HCG300	420			DIED		
66	1993	1	308	310	F	VI	RR		YES	1	0	51816	77
67	1993	1	400	326	F	V	HCG300	430	YES	1	34.5	101677	39
68	1993	1	310	313	F	V	HCG300	430	YES	2	34.5	73325	30
69	1993	1	320	325	F	V	OVA	421	YES	2	36	60615	98
70	1993	1	296	296	F	VI	RR		YES	1	0	47905	46
71	1993	1	250	280	F	V	OVA	455	NO				
72	1993	1	256	295	F	V	OVA	394	YES	1	33.5	68437	81
73	1993	1	221	273	F	V	OVA	400	NO				
74	1993	2	150	255	M	VI	OVA						
75	1993	2	170	257	M	VI	OVA						
76	1993	2	200	272	M	VI	OVA						
77	1993	2	225	272	F	V	OVA	446	NO				
78	1993	2	160	256	F	V	OVA	477	YES	2	33	96789	76
79	1993	2	170	262	F	V	OVA	407	YES	1	34.8	2933	10
80	1993	3	200	261	F		HCG1000	434	YES	1	44	UN	72
81	1993	3	150	255	F		HCG1000	446	YES	1	44	13687	3
82	1993	3	200	271	F		HCG1000	455	YES	1	44	45950	42
83	1993	3	150	247	F		HCG1000	449	NO				
84	1993	3	175	248	F		HCG1000	458	YES	1	44	49861	UN
85	1993	3	150	258	F		HCG1000	523	YES	1	44	7821	2
86	1993	3	75	237	F		NIL						
87	1993	3	100	220	F		NIL						
88	1993	3	100	240	M		HCG1000						
89	1993	3	100	220	M		HCG1000						
90	1993	3	134	242	F		HCG1000	428	NO				
91	1993	3	173	256	F		HCG1000	440	YES	1	33	40084	75
92	1993	3	154	245	F		HCG1000	440	YES	1	33.5	23464	24
93	1993	3	161	250	F		HCG1000						
94	1993	3	211	273	M		HCG1000						
95	1993	3	356	328	F		HCG1000	431	YES	1	33	139806	99
96	1993	3	200	275	F		HCG1000	425	NO				
97	1993	3	234	281	F		HCG1000	520	YES	1	33.3	60615	69
98	1993	3	181	264	F		NIL	<200					
100	1993	3	200	270	F		HCG1000	474	YES	1	33.3	64526	37
101	1993	3	256	285	F		HCG1000	388	NO				
102	1993	3	199	265	F		HCG1000	418	YES	1	33	5866	58
103	1993	3	314	316	F		HCG1000	449	YES	1	33	61593	86
104	1993	3	203	275	F		HCG1000	428	YES	1	33.5	7831	60

105	1993	3	324	320	M		HCG1000						
106	1993	3	266	295	M		HCG1000						
107	1993	3	263	288	F		HCG1000	477	YES	1	32.5	118298	81
108	1993	3	182	258	F		HCG1000	471	YES	1	33	40084	62
109	1993	3	140	248	F		HCG1000	378	NO				
110	1993	3	204	269	M		HCG1000						
111	1993	3	154	247	F		HCG1000	428	YES	1	33.5	28352	58
112	1993	3	207	277	F		HCG1000	462	YES	1	33	32263	77
113	1993	3	143	253	M		HCG1000						
114	1993	3	237	307	M		HCG1000						

APPENDIX A3 Summary hormone induction trials with mulloway *Argyrosomus hololepidotus* at the Port Stephens Research Centre 1992 to 1994

Ref no.	Year	Month	Weight (g)	TL (mm)	Sex	Hormone treatment	Oocyte (um)	Ovulation	Batch	Latent period (h)	No. of eggs	Fert (%)
1	1992	2	4870	744	UN							
2	1992	2	4000	714	UN							
3	1992	2	6380	790	M		VI					
4	1992	2	6060	817	UN							
5	1992	2	5400	779	UN							
6	1992	2	5650	761	UN							
7	1992	2	6470	833	UN							
8	1992	2	4190	717	UN							
9	1992	2	4440	740	UN							
10	1992	2	6780	807	M		VI					
11	1992	2	4900	768	UN							
12	1992	2	2000	565	UN							
13	1992	2	6700	865	UN							
14	1992	2	4360	777	UN							
15	1992	2	5640	775	UN							
16	1992	2	4800	767	UN							
17	1992	2	6050	807	F		II					
18	1992	2	5870	846	M		VI					
19	1992	2	5200	775	M		VI					
20	1992	2	5920	844	M		VI					
21	1992	2	5680	UN	UN							
22	1992	2	6130	783	M	HCG250	VI					
23	1992	2	10720	1005	F	HCG1000	515	YES	1	32	1042000	70
24	1992	2	9800	982	M	HCG250	VI					
25	1992	2	10000	992	F	HCG1000	477	YES	1	34	900000	4
26	1993	2	7300	820	F	HCG1000	559	YES	1	44	61593	0
27	1993	2	6000	840	?							
28	1993	2	6200	810	F							
29	1993	2	6600	770	M	HCG250						
30	1993	2	6700	837	?							
31	1993	2	5600	800	?							
32	1993	2	4400	740	?							
33	1993	2	7600	890	M	HCG250						
34	1993	2	6100	870	?							
35	1993	2	7600	933	?							
36	1993	2	5750	810	?							
37	1993	2	6400	825	M	HCG234						
38	1993	2	6500	860	F							
39	1994	2	9100	940	M							
40	1994	2	10540	993	M							
41	1994	2	9600	925	M							
42	1994	2	7700	885	M							
43	1994	2	8890	904	F	HCG1000	592	YES	1	42	739722	0 TO 7
44	1994	2	9040	969	M	HCG500						
45	1994	2	6520	866	?							
46	1994	2	8640	942	M							
47	1994	2	12550	1053	M							
48	1994	2	8500	996	M							

49	1993	2	14000	1130	F	HCG1000	662	YES	1	36	1935956	1 TO 8
50	1993	2	2300	643	M	HCG250	VI					
51	1993	3	14300	UN	F	HCG1000	800?	YES	2	2 TO	377574	0
52	1994	1	5340	849	F	HCG1000	569	YES	1	34	617230	45

Mulloway broodstock held at Port Stephens in 50 00L tank

Ref no.	Year	Month	Weight (g)	TL (mm)	Sex	Stage
1	1991	2	3500	770		
2	1991	2	2500	665		
3	1991	2	3200	720		
4	1991	2	4650	795		
5	1991	2	3350	800		
6	1991	2	6350	805		
7	1991	2	4250	775		
8	1991	2	5150	815		
9	1991	2	4750	805		
10	1991	2	6100	870		
11	1991	2	3450	730		
12	1992	1	6600	900	m	VI
13	1992	1	5640	847	f	II
14	1992	1	6700	878	m	V
15	1992	1	6500	855	m	VI
16	1992	1	7500	895	m	V
17	1992	1	6750	880	?	
18	1992	1	7650	912	m	VI
19	1992	1	5940	832	m	V
20	1992	1	6320	900	?	
21	1992	1	6830	872	?	
22	1992	1	6060	830	?	
23	1993	1	11000	1040	F	II
24	1993	1	11000	1028	F	II
25	1993	1	9800	997	F	II
26	1993	1	10150	994	F	II
27	1993	1	8500	946	F	II
28	1993	1	8500	944	?	
29	1993	1	10200	1020	M	VI
30	1993	1	9200	950	M	
31	1993	1	10000	1030	M	
32	1993	1	10600	995	M	
33	1993	1	7400	900	M	
34	1993	1	3750	720	M	
35	1993	1	9660	957	F	515
36	1994	2	UN	980	F	
37	1994	2	7380	900	M	
38	1994	2	10050	1045	F	
39	1994	2	9750	1024	M	
40	1994	2	4010	792	M	
41	1994	2	5110	809	?	

APPENDIX B List of cultured fish larvae in which initial swim bladder inflation has been studied or swim bladder dysfunction has been recorded

Family	Species	Common name	Reference
Moronidae	*Dicentrarchus labrax*	European sea bass	Chatain 1989
	Morone saxatilis	Striped bass	Hadley et al. 1987
	M. chrysops x M. saxatilis	Palmetto or sunshine bas	Chapman et al. 1988
Percichthyidae	*Macquaria novemaculeata*	Australian bass	Battaglene @ Talbot 1990
Serranidae	*Lateolabrax japonics*	Japanese sea bass	Hayashida et al 1984
Sparidae	*Sparus auratus*	Gilthead sea bream	Chatain 1989
	Acanthopagrus cuvieri	Sobaity	Al-Abdul-Elah et al. 1983b
	Pagrus auratus = (P.major)	Red sea bream or snappe	Foscarini 1988
Centrarchidae	*Micropterus salmoides*	Largemouth bass	Johnston 1953
	Micropterus dolomieni	Smallmouth bass	Spoor 1984
Mugilidae	*Mugil cephalus*	Sea mullet	Nash and Kuo 1975
Cichlidae	*Sarotherodon mossambica*	Tilapia	Doroshev et al. 1981
Carangidae	*Seriloa quinqueradiata*	Yellowtail kingfish	Kitajima et al.1994
	Seriola aureovittata	Amberjack	Kitajima et al.1994
Gadidae	*Boreogadus saida*	Polar cod	Aronovich et al. 1975
Latrididae	*Latris lineata*	Striped trumpeter	Ruwald et al. 1991
Clupeidae	*Brevoortia tyrannus*	Atlantic menhaden	Hoss et al. 1989
	Brevoortia patronus	Gulf menhaden	Hoss and Phonlor 1984
	Engraulis japonicus	Japanese anchovy	Uotani 1973
	Clupea harengus	Atlantic herring	Blaxter and Batty 1984
	Engraulis mordax	Northern anchovy	Hunter and Sanchez 1976
Percidae	*Perca fluviatilis*	Redfin	Ribi 1992
	Stizostedion vitreum	Walleye	Barrows et al. 1993
Sciaenidae	*Argyrosomus hololepiddotus*	Mulloway or kob	Battaglene and Talbot 1994
	Atractoscion nobilis	White sea bass	Orhun 1989
	Sciaenops ocellata	Red drum	Soletchnik et al. 1988
Sillaginidae	*Sillago ciliata*	Sand whiting	Battaglene et al. 1994
	Sillago maculeata	Trumpeter whiting	Battaglene unpublished data 1993
Salmonids	*Salvelinus alpinus*	Arctic charr	Wallace and Aasjord 1984
Soleidae	*Solea solea*	Dover sole	Boulhic and Gabaudan 1992
Scopthalmidae	*Scophthalmus maeoticus*	Black sea turbot	Spectorova and Doroshev 1976
Cyprinidae	*Cyprinus carpio*	Common carp	Korwin-Kossakowski 1988
Acipenseridae	*Huso huso*	Beluga	Tsvetkov and Sbikin 1983

APPENDIX C1 Summary of Australian bass larval rearing trials conducted at Port Stephens Research Centre 1989-1992

Reference	Date hatched	Tank R=10000L L=2000L	Stocking density /L	Length of trial days	Swim bladder inflation 12 DAH %	Survival %	Mean temperature °C (SD)	First feed Rotifers		First feed Artemia	
								Age DAH	Size mm	Age DAH	Size mm
A1	19-5-89	R3	50	19	67	50	19.2(1.0)	5	4.4	16	5.5
A2	28-5-89	R2	50	10	98	50	19.1(0.8)	6	4.4	NA	NA
A3	24-6-89	R3	50	13	100	63	19.1(1.0)	6	4.6	NA	NA
A4	24-6-89	R1	50	14	100	20	18.0(1.6)	6	4.5	NA	NA
A5	24-6-89	R2	50	40	100	25	17.6(1.3)	6	4.5	13	5.5
A6	30-7-89	R3	50	16	90	50	18.8(0.7)	5	4.3	12	5.1
A7	25-5-90	R3	50	35	60	0	18.6(1.2)	7	4.6	12	4.4
A8	25-5-90	R2	50	18	90	50	19.0(0.7)	7	4.4	12	4.6
A9	22-6-90	R3	50	20	60	25	18.2(1.1)	NA	NA	12	4.2
A10	22-6-90	R2	50	20	70	50	17.8(1.2)	8	4.5	16	5
A11	29-6-91	L4	36	19	60	22.4	19.8(2.1)	8	4.6	16	5.7
A12	29-6-91	L5	36	19	30	5.5	20.0(2.5)	8	4.6	16	4.8
A13	20-7-91	L5	100	21	90	7.2	20.7(1.2)	10	4.2	19	5.2
A14	20-7-91	L4	100	21	100	11.9	20.6(1.5)	10	4.3	19	5.3
A15	6-6-92	L6	49	23	100	0	21.5(0.8)	NA	NA	7	4.2
A16	13-7-92	L1	100	14	90	33	19.9(0.5)	10	4.6	NA	NA
A17	13-7-92	L2	100	14	70	60	21.2(1.0)	10	4.5	NA	NA
A18	13-7-92	L6	100	14	20	?	20.6(1.3)	10	4.6	NA	NA
A19	25-6-92	L1	50	12	100	80	19.9(1.4)	NA	NA	9	4.6
A20	6-6-92	R3	72	18	70	8.9	21.5(0.8)	NA	NA	7	4.2
A21	6-6-92	R2	61	18	70	40.8	21.6(0.8)	NA	NA	7	4.4
A22	6-6-92	L1	93	18	90	32.2	20.1(1.5)	NA	NA	7	4.5

APPENDIX C1(cont)

Reference	Rotifer schedule n/ml ( )=no. feeds/day (=1 unless otherwisw stated)				Artemia schedule n/ml ( )=no. feeds/day (=1 unless otherwisw stated)					
	0-5	6-10	11-15	16-20	10-15	16-20	21-25	26-30	31-35	36-40
A1	10	15	15(2)	10	0	2	NA			
A2	0	10	NA							
A3	0	10	10	NA	NA					
A4	0	10	15(2)	NA	NA					
A5	0	10	15(2)	15(2)	1	0.8	1	2	3	5
A6	0	10(2)	10(2)	NA	NA					
A7	0	12	15	12	0.5	1.5	0.5	0.2	0.8	NA
A8	0	10	15	10	0.6	0.6	NA			
A9	NA				1	1.5	NA			
A10	0	15	15	15	0	1	NA			
A11	0	15	10	10	0	0.9	NA			
A12	0	10	15	10	0	0.9	NA			
A13	0	15	15	15	0	1	NA			
A14	0	15	15	15	0	1	NA			
A15	NA	NA	NA	NA	0.8(2)	0.8(2)	0.8(2)	NA		
A16	0	10	10	NA	NA					
A17	0	10	10	NA	NA					
A18	0	10	10	NA	NA					
A19	NA				0.5	NA				
A20	NA				0.8(2)	0.5				
A21	NA				0.8(2)	0.5				
A22	NA				0.8(2)	0.8(2)				

APPENDIX C2 Summary of snapper *Pagrus auratus* larval rearing trials at Port Stephens Research Centre 1990 to 1993

Reference	Date hatched	Tank R=10000L L=2000L	Stocking density /L	Length of trial days	Swim bladder inflation 12 DAH	Survival %	Mean temperature °C	First feed Rotifers		First feed Artemia		First feed Other food
								Age DAH	Size mm	Age DAH	Size mm	Age DAH
S1	16-11-90	R3	1	50	70	13	23.9(2.9)	4	3.2	23	6.9	33
S2	22-11-91	R2	0.6	25	69	24	21.1(1.3)	5	UN	21	5.3	40
S3	5-11-91	R3	3.3	43	30	23	20.9(1.5)	5	UN	19	5.6	41
S4	16-10-92	R3	24	25	60	18.1	18.9(1.4)	5	3.4	19	5.7	NA
S5	18-10-92	L6	30	75	60	UN	20.4(1.5)	6	4	20	5.5	34
S6	18-10-92	L3	30	62	80	11	20.7(1.4)	6	3.4	19	6.4	34
S7	16-10-92	L1	29	19	30	10.5	18.8(1.5)	5	3.6	23	6.9	NA
S8	27-8-93	L1	17	35	83	8.7	19.8(0.8)	4	3.3	20	6.5	34
S9	19-9-93	L2	24	46	87	9.4	20.9(0.8)	5	3.3	25	6.5	27
S10	21-10-93	L3	31	26	90	0.8	21.1(0.7)	5	3.3	18	5.8	35
S11	13-11-93	L1	13	42	UN	33.3	22.7(1.3)	6	3.5	18	UN	24

Reference	Rotifer schedule n/ml ( )=no. feeds/day (=1 unless otherwise stated)					Artemia schedule n/ml ( )=no. feeds/day (=1 unless otherwise stated)						
	0-5	6-10	11-15	16-20	21-25	16-20	21-25	26-30	31-35	36-40	41-45	46-50
S1	12	10	11	10	7	0.4	0.8	0.8	0.8	0.5	0.5	0.5
S2	10	10	10	10	5	0	0.4	0.7	0.9(3)	0.9(3)	0.7(3)	0.2(2)
S3	10	12	16	15	12	0.2	0.6(3)	0.8(3)	2.2(6)	3.1(6)	2.5(6)	1.8(6)
S4	5	7	7	8	3	0.2	0.5					
S5	0	8(2)	7(2)	5(2)	5(2)	0	0.4(2)	0.9(3)	0.9(3)	0.7(3)	0.3	
S6	0	7	8	5	4	0.5(2)	1.5(4)	1.0(3)	0.3	0.5		
S7	8	6	8	8	NA	NA						
S8	10	12	15	15	15	0	0.3	0.5	0.7(2)	0.9(20	0.3(2)	0.2
S9	15	13	13	16	8	0.6(3)	0.9(3)	0.3(3)	0.5(5)	0.5(5)	0.5(5)	0.5(5)
S10	5	13	8	15	13	0.4	0.8(2)	1.2(3)	2.5(3)	3.0(3)	3.8(3)	3.8(4)
S11	5	10	15	15	15	0.4	0.9(2)	1.8(4)	4.4(5)	3.6(4)	3.8(4)	3.4(4)

APPENDIX C2 (cont)

Reference	Other food schedule					Disease			
	31-40	41-50	51-60	61-70	71-80	Age DAH	Size mm	Type	Treatment
S1	squid pilchard	pilchard				NA			
S2	pilchard	pilchard				NA			
S3	pilchard	pilchard	pilchard pellets	pilchard pellets	pilchard pellets	NA			
S4	pilchard whiting larvae	pilchard	pilchard pellets	pilchard pellets	pilchard	NA			
S5	NA					NA			
S6	pilchard prawn skretting	pilchard prawn skretting	pilchard prawn skretting	pilchard prawn skretting	bivalve artemia skretting	26 53 61 75	7.4 22 25 30.6	blue spot blue spot blue spot +bacteria	oxytet enroflo enroflo oxytet
S7	ML250	ML250 ML400	ML400 ML800			54	23	blue spot	oxolinic
S8	ML250 ML400	ML400 ML800	ML800 C1	ML800 C1	C1 C2	32	8.5	blue spot	oxolinic
S9	ML250 ML400	ML400 ML800	ML800 C1	ML800 C1		22	5.6	blue spot	oxolinic
S10	ML250 ML400	ML250 ML400	ML400 ML800	ML400 ML800		15	5.4	blue spot	oxolinic

APPENDIX C3 Summary of sand whiting *Sillago ciliata* larval rearing trials conducted at Port Stephens Research Centre 1992-1994

Reference	Date hatched	Tank R=10000L L=2000L	Stocking density /L	Length of trial days	Harvest	Survival %	Mean temperature °C (SD)	First feed Rotifers		First feed Artemia		First feed Other food
								Age DAH	Size mm	Age DAH	Size mm	Age DAH
W1	9/2/92	R2	100	31	92	<0.0001	22.9(1.7)	3	UN	18	5.9	NA
W2	15/1/93	L1	60	50	1097	0.9	24.5(1.3)	4	2.8	26	9.5	43
W3	18/3/93	L3	75	20	10000	6.7	21.7(0.9)	3	2.8	NA	NA	NA
W4	18/3/93	L1	75	20	30000	20	21.6(0.9)	3	2.9	NA	NA	NA

	Rotifer schedule n/ml ( )=no. feeds/day (=1 unless otherwise stated)				Artemia schedule n/ml ( )=no. feeds/day (=1 unless otherwise stated)							
	0-5	6-10	11-15	16-20	10-15	16-20	21-25	26-30	31-35	36-40	41-45	50-55
W1	5SS	WP 5	10	10	0	0.2	0.2	0.4(2)	NA			
W2	6SS	10	10	10(2)	0	0	0	1.2(3)	2.4(3)	2.9(3)	1.6(2)	0
W3	8SS	10SS	10	10	NA							
W4	10SS	10SS	10	10	NA							

SS = small strain rotifer

APPENDIX C4 Summary of mulloway *Argyrosomus hololepidotus* larval rearing trials conducted at Port Stephens Research Centre 1992-1994

Reference	Date hatched	Tank R=10000L L=2000L	Stocking density /L	Length of trial days	Swim bladder inflation 12 DAH	Surviva %	Mean temperature °C	First feed Rotifers		First feed Artemia		First feed Other food
								Age DAH	Size mm	Age DAH	Size mm	Age DAH
M1	14/2/92	L1	50.0	25	87	7.3	23.4	3	3.0	12	4.2	NA
M2	14/2/92	R3	50.0	26	87	0.0	22.7	3	2.9	12	4.3	39
M3	6/2/93	L2	5.0	46	100	0.6	22.6	3	2.7	13	4.5	39
M4	23/1/94	L3	22.8	16	100	54.9	24.0	4	2.8	12	4.6	NA
M5	23/1/94	L1	51.3	44	100	2.5	24.0	4	2.8	12	4.4	24
M6	23/1/94	L2	25.6	51	100	3.3	23.8	4	2.8	12	4.5	24
M7	23/1/94	L6	17.6	44	100	6.4	23.9	4	2.7	12	4.4	24

Reference	Rotifer schedule n/ml ( )=no. feeds/day (=1 unless otherwise stated)				Artemia schedule n/ml ( )=no. feeds/day (=1 unless otherwisw stated)						
	0-5	6-10	11-15	16-20	10-15	16-20	21-25	26-30	31-35	36-40	41-45
M1	6	6	5	0	0.2	0.6 (3)	0.8 (4)	NA			
M2	6	6	6	0	0.2	0.4 (2)	0.6 (2)	0.3 (3)	0.6 (3)	0.4 (2)	30g adults
M3	10	10	7	7	0.75	1.0 (2)	1.2 (2)	1.4 (2)	2.6 (2)	2.2 (2)	NA
M4	5	10	12	12	0.4 (2)	0.4 (2)	NA				
M5	5	10	12	12	0.4 (2)	0.6 (2)	1.2 (6)	1.8 (6)	1.8 (6)	1.2 (6)	0.8 (3)
M6	5	10	12	12	0.4 (2)	0.6 (2)	1.2 (6)	1.8 (6)	1.8 (6)	1.2 (6)	0.8 (3)
M7	5	10	12	12	0.4 (2)	0.6 (2)	1.2 (6)	1.8 (6)	1.8 (6)	1.2 (6)	0.8 (3)

APPENDIX C4 (cont)

Reference	Other food schedule						Disease			
	21-30	31-40	41-50	51-60	61-70	71-80	Age DAH	Size mm	Type	Treatment
M1	NA						NA			
M2	0	pilchard	pilchard skretting	pilchard skretting	pilchard skretting	pilchard skretting	NA			
M3		pilchard	pilchard RBD	prawn RBD	pilchard	pilchard aqua feed	NA			
M4	NA						NA			
M5	ML250 LM400	ML250 ML400 ML800	ML400 ML800				33	11	white spot	oxolinic acid
M6	ML250 LM400	ML250 ML400 ML800	ML400 ML800				33	14.3	white spot	oxolinic acid
M7	ML250	ML250	ML400				13	4.6	increase in	oxolinic acid

BWFCRS: MARINE FISH BREEDING REARING DATA

SPECIES:
SHEET NO.:
TANK NO.:

NO. STOCKED:
NO.HARVESTED:

DATE STOCKED:
DATE HARVESTED:

Days		Water Quality					Water Exch		Food Count		Food Added			Larvae				
Date	D	pH	DO	°C	Sal	NH^3	Flow	Bio	Rot	Art	Rot	Art	Oth	TL	±	SB	Fed	Comment

APPENDIX D. Data sheet used to record daily larval rearing details

APPENDIX E

List of publications arising from research reported in the thesis.

Battaglene, S.C., in review. Induced ovulation of captive and wild snapper *Pagrus auratus* (Sparidae) using hCG, LHRHa and Ovaprim. New Zealand Journal of Marine and Freshwater Research.

Battaglene, S.C., in review. Induced ovulation of sand whiting, *Sillago ciliata*. Asian Fisheries Science.

Battaglene, S.C., 1994. Developments in hatchery production of mulloway *Argyrosomus hololepidotus*. Australian Society for Fish Biology, 21 st Annual Conference, Canberra, ACT 2-3 September 1994. (Abstract only).

Battaglene, S. C. and Allan, G. L., in press. Development of hatchery techniques for snapper, *Pagrus auratus*, in Australia. In: Proceedings of the Third Asian Fisheries Forum, Singapore 1992.

Battaglene, S. C. and Allan, G. L., 1990. Australian bass - a potential winter crop for the prawn farming industry in NSW and Southern Queensland. Australian Fisheries, 49:31-32.

Battaglene, S.C., and Bell, J.D. 1991. Aquaculture prospects for marine fish in New South Wales, Fishnote DF/6, NSW Agriculture & Fisheries, Sydney.

Battaglene, S. C. and Selosse, P., inpress. Hormone-induced ovulation and spawning of the catadromous Australian bass *Macquaria novemaculeata* (Percichthyidae). Aquaculture and Fisheries Management.

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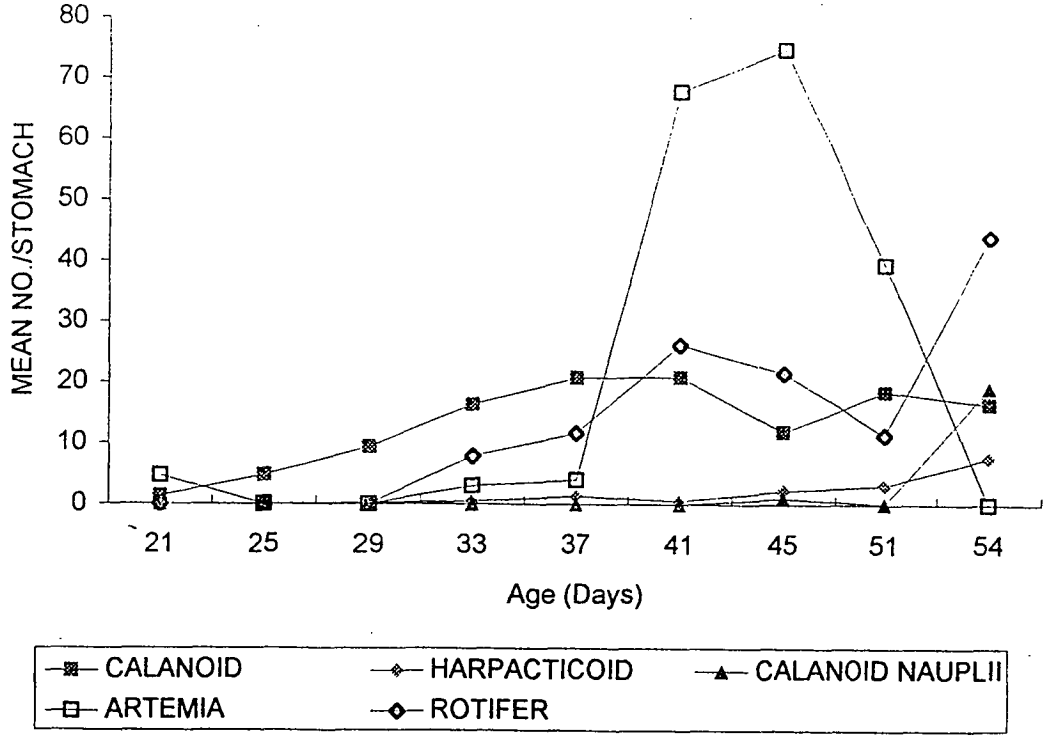
APPENDIX F

Additional information provided to support results presented in Chapter 5.

The strain of *Artemia* used was from the Great Salt Lakes (Red Jungle brand). *Artemia* were hatched in 200 L tanks and added to the ponds as newly hatched nauplii without enrichment. The stomach contents of Australian bass larvae sampled prior to the addition of *Artemia* is given in Figure 1. The composition of plankton in the ponds prior to stocking of *Artemia* is presented in Figure 2.

Figure 1 Mean number of items found in Australian bass stomachs (n>10) reared in 0.01 ha ponds covered in a green house. Fish sampled before the addition of *Artemia*.



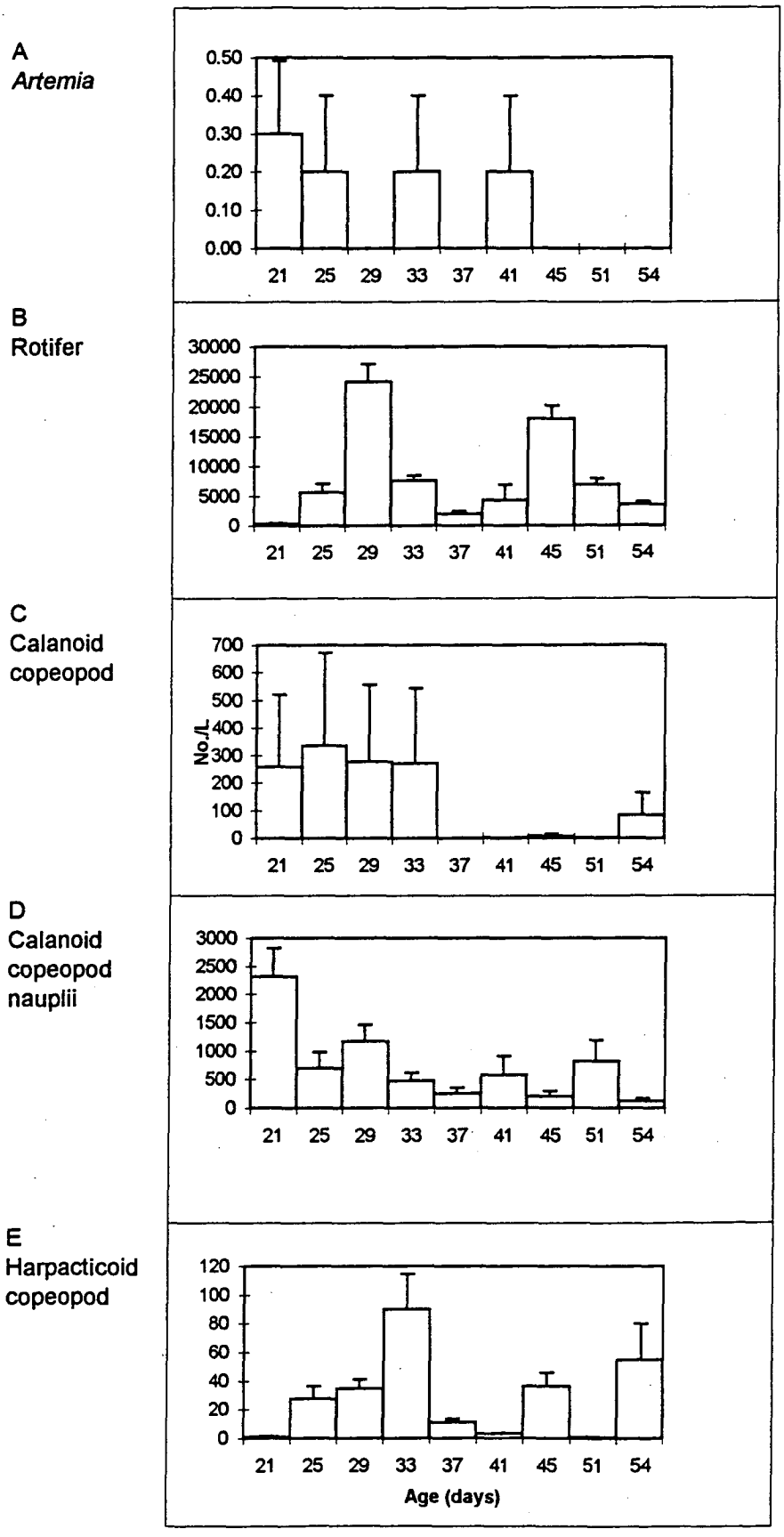


Fig. 2 Histograms of zooplankton concentrations in four green house ponds during Australian bass rearing trials. Values represent means, error bars are standard errors.